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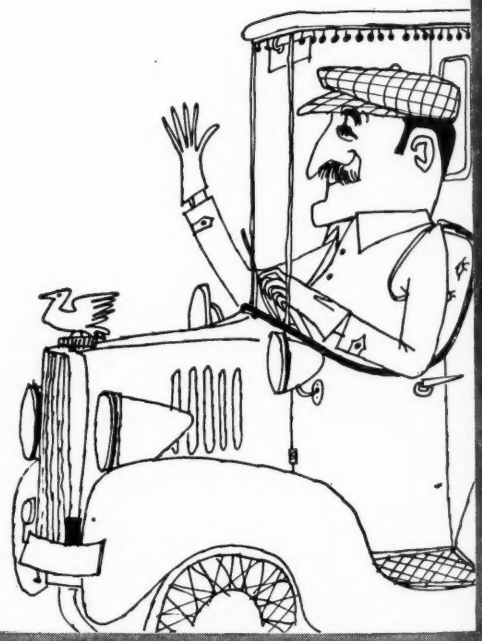

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The Journal of Allergy

VOL. 27 MARCH, 1956 NO. 2

Original Articles

STUDIES ON THE HOUSE DUST ALLERGEN(S)

I. THE RELATIONS BETWEEN ANTIGENIC AND ALLERGENIC COMPONENTS

EDNA M. FOLLENSBY, PH.D., FRANCIS C. LOWELL, M.D., AND IRVING W. SCHILLER, M.D., BOSTON, MASS.

THE source of the skin-reactive components present in extracts made from house dust, cotton, or kapok, especially after aging, has engaged the attention of many workers since house dust was first described by Cooke[1] as one cause of symptoms in patients with rhinitis and asthma. The object of the experiments reported herein was to identify in such materials those components inducing complement-fixing and precipitating antibody in rabbits and to study the relation between these and that giving rise to skin reactions in clinically dust-sensitive persons. If successful, such a step might facilitate later attempts to trace the source of such antigenic and allergenic materials as are present in them.

MATERIALS AND METHODS

Dust Extracts.—Extract E, used as the principal antigen, was a lyophylized light brown powder obtained by precipitation with acetone and dioxane from an aqueous extract of old cotton mattress stuffing.* One gram of the powder was readily soluble in 10 ml. of physiologic saline to give a 1:10 dilution, a somewhat viscid, dark brown solution; clear-cut skin reactions were obtainable in selected persons with dilutions of 1:40,000 or higher.

Extract C was a purified fraction prepared from a house dust extract kindly supplied by Dr. D. H. Campbell.[2]

Adjuvant Mixture.—Sterile technique was used in preparing the adjuvant mixture, which contained one part of Falba, 1.2 parts of Bayol F containing

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Supported by a grant from the United States Public Health Service.

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*We are indebted to Dr. Samuel M. Gordon, Endo Products, Inc., for a liberal supply of this extract, for a generous quantity of old mattress stuffing, the starting material for extract E, and an extract prepared from freshly ginned cotton.

20 mg./100 ml. of heat-killed, dried *Mycobacterium butyricum,* and 1.8 parts of a glycerinated extract E (1 gm./60 ml.). This mixture was freshly prepared each time injections were given.

Antisera.—The schedule followed in immunizing the rabbits is shown in Table I. Two groups were injected with extract E. Positive results in complement fixation tests were observed with the antisera from the first group of five rabbits (Nos. 1 to 5), but the precipitin reactions were weak and the sera were not useful in agar diffusion tests.

TABLE I. IMMUNIZATION OF RABBITS WITH DUST EXTRACT E

DATE	RABBIT NO.	ROUTE	NUMBER OF SITES INJECTED	TOTAL EXTRACT* INJECTED (ML.)	BLEEDING NO.
		First Group			
7/31/53	1-5	Subcutaneous	2	1.0†	
9/16	1-5	Subcutaneous	2	1.0†	
10/22					1
11/6	1-5	Intracutaneous	6	1.2-2.0	
12/23	1-5				2
3/16/54	1-5	Subcutaneous	1	1.0 of 1:400	
3/18	1-5		1	1.0 of 1:200	
3/23	1-5				3
		Second Group			
11/12/53	6-11	Intracutaneous	10	2.6-3.0**	
11/13	12-15	Intracutaneous	10	3.0-4.0**	
12/23	6, 7, 8, 13, 14, 15				1
12/29	9-12				1
3/16/54	6-14	Subcutaneous	1	1.0 of 1:400	
3/18	6-14	Subcutaneous	1	1.0 of 1:200	
3/19	6-14	Subcutaneous	1	1.0 of 1:120	
3/23	6, 7				2
3/24	8, 9, 10, 13				2
3/25	11, 12, 14				2
4/14	10				3
5/18	14	Subcutaneous	1	1.0 of 1:400	
5/21	14	Subcutaneous	1	1.0 of 1:120	
5/26	14				3
10/20	6-9	Subcutaneous	1	0.2 of 1:400	
10/21	6-9	Subcutaneous	1	0.35 of 1:400	
10/22	6-9	Subcutaneous	1	1.0 of 1:400	
10/25	6-9	Subcutaneous	1	1.0 of 1:400	
10/27	6-9	Subcutaneous	1	1.5 of 1:400	
11/2	6-9				3
11/15	11-13				3
11/23	6-9				4

*In saline solution except where indicated.
†Extract incorporated in adjuvant (see text).

After preliminary bleeding (normal sera) rabbits 6 to 15 were given extract E with adjuvant intracutaneously, followed later by two series of subcutaneous injections of extract diluted 1:120, 1:200, or 1:400 in physiologic saline. The antisera obtained from these rabbits were used satisfactorily in agar diffusion tests.

A total of forty-seven immune bleedings was obtained from the fifteen rabbits, comprising fifteen first bleedings, fourteen second bleedings, fourteen third bleedings, and four fourth bleedings.

Complement Fixation.—The 50 per cent unit of 1:100 guinea pig C′ was determined with the Coleman Junior spectrophotometer. Four units and 0.2 ml. of a 1 per cent suspension of washed sheep cells sensitized with 2 units of rabbit antisheep lysin were used in the fixation tests. The fixing and final volumes were 0.3 and 0.5 ml., respectively. The mixture of complement, antiserum, and antigen was incubated for eighteen to twenty-four hours at 4° to 6° C. and again, after the addition of the lytic system, for thirty minutes at 37° C. The diluent for all reagents was Veronal buffer.[3] The maximal dose of both antiserum and antigen was one-fourth the anticomplementary dose which, for the antisera, ranged from 0.1 ml. of 1:10 to 0.1 ml. of 1:100; for the dust antigen it was 0.1 ml. of a 1:4,000 dilution. The antigen was usually diluted in twofold steps to 1:64,000 or higher.

Agar Diffusion.—Agar diffusion tests, using 0.6 per cent agar containing 0.4 per cent phenol, were done in tubes by both single diffusion and double diffusion techniques.[4] With the same concentration of agar, tests were also done in plates,[5] using Petri dishes 55 to 60 mm. in diameter. Wells were formed with penicillin cups 15 to 30 mm. apart. For comparison of the reactivity of two antisera with one antigen or of two antigens with one antiserum, the three cups were carefully set at the apices of an equilateral triangle. In the majority of the tests with agar in tubes or in plates, the antisera were diluted 1:2, although more dilute or undiluted serum was also used in some instances. The diluent for antiserum and antigen was 0.85 per cent sodium chloride solution containing 0.4 per cent phenol.

RESULTS

Skin Reactions to Extract E in Immunized Rabbits.—The rabbits did not react with either general or local manifestations to the subcutaneous stimulating injections of extract E. Intracutaneous tests, which were read at twenty minutes and again at twenty-four and forty-eight hours, were done at two different dates on some of the rabbits in the second group (Table II). There were no reactions seen at twenty minutes. In the earlier tests, done four weeks after the initial stimulating injections, all of eight rabbits showed some degree of reaction at twenty-four or forty-eight hours to a 1:400 dilution of extract. In the later tests, done in seven of these rabbits, all gave some reaction in twenty-four to forty-eight hours with the extract diluted 1:4,000.

In Vitro Tests With Antisera and Dust Extract E.—All of forty-seven antisera obtained from the injected animals contained precipitins as determined by the interfacial technique. The sera from the five rabbits in the first group produced somewhat less precipitate than the sera from the ten rabbits in the second group and, as would be expected, more precipitate was seen in the later bleedings from all animals.

Forty-five of the forty-seven antisera were tested for their capacity to fix complement with the homologous extract E. Only three antisera failed to fix, but these were so anticomplementary that they could not be used in dilu-

tions lower than 1:80 or 1:100. The remaining forty-two antisera fixed completely with extract diluted 1:4,000 and some with extract diluted 1:64,000 (Table II).

TABLE II. SEROLOGICAL AND SKIN TESTS IN RABBITS WITH DUST EXTRACT E

ANTI-SERUM NO.	DILUTION OF ANTI-SERUM*	TITER† BY COMPLEMENT FIXATION (PER CENT FIXATION)			AGAR DIFFUSION TECHNIQUE (MAXIMAL NO. OF BANDS)		DEGREE OF SKIN REACTIVITY‡ IN IMMUNIZED RABBITS			
							4/14/54		12/13/54	
		100	75	50	BY PLATE	BY TUBE	4,000	400	4,000	400§
6_1	30	10,000				2				
6_2	40	32,000			4	4	0	++	+	0‖
	100	16,000								
6_3					5					
6_4					0					
7_1	50	10,000	40,000	100,000						
7_2	40	32,000			2	2				
7_3					4		++	+++	+	++
7_4					1					
8_1	100	10,000								
8_2	40	32,000		64,000	2	3				
8_3					3		+	+++	-	+++
8_4					3					
9_1	100	32,000	64,000	128,000						
9_2	50	16,000		32,000	2	2				
9_3					3		+	+++	+	+
9_4					3					
10_1	100	32,000	64,000	128,000						
10_2	40	32,000	64,000		2	3				
	100	32,000		64,000						
11_1	25	16,000		32,000						
11_2	70	64,000		128,000	3	3	0	+++	±	+
11_3					3					
12_1	25	16,000								
12_2	70	32,000			2	2				
	60	16,000		32,000			+	+++	±	++
12_3					2					
13_1	100	40,000				2				
13_2	40	32,000	64,000		2	3				
	100	16,000	32,000				0	++	+	+++
13_3					2					
14_1	10	48,000		64,000						
14N¶	10			8,000			0	++		
14_2	50	32,000			2	2				
	70	16,000	32,000							
14_3					4	2				
15_1	10	64,000								
15N¶	10	32,000	64,000							

*Expressed as reciprocal of serum dilution.
†Expressed as reciprocal of antigen dilution.
‡Read at twenty-four hours.
Erythema less than 10 mm., no edema = ±.
Erythema 10 mm. or more, no edema = +.
Erythema 10 mm. or more, slight edema or hemorrhagic area = ++.
Erythema 10 mm. or more, definite edema = +++.
§Expressed as reciprocal of dilutions of dust extract E.
‖Test done twice with negative result.
¶Normal serum.

Preimmunization (normal) sera from animals 14 and 15 had some fixing capacity for dust extract. Animal 15, in a dilution of 1:10, fixed almost as well as the immune bleeding in the same dilution. Neither of these produced precipitate in the interfacial test. The preimmunization sera from the remaining eight animals did not fix complement in doses of 0.1 ml. of 1:20 or 1:30 dilution of serum.

With the small amount of C′ used, no prozones were observed in any of these tests for fixation, perhaps because the range of antigen dilution was of necessity limited by the anticomplementary nature of extract E. With the use of 12 units of C′, permitting a maximal antigen concentration of 1:400, there was a short prozone with three antisera with the weakest of the three dilutions of serum tested. Only one zone of fixation was observed although, as judged by agar diffusion tests to be described below, most of the antisera contained antibody for two or more antigens. It was felt, therefore, that analysis of the antigenic composition of the extract by complement fixation would require very extensive testing and might not be rewarding.

Twenty-six immune sera were tested in tubes[3] or plates[4] with extract E by the agar diffusion technique. All the immune sera gave one or more bands and the third bleeding from animal No. 6 (serum 6_3) gave five, the greatest number observed with any of the sera. The results are shown in Table II. It is interesting to note that sera from the third bleedings from rabbits 11, 12, and 13 produced bands, although these rabbits had not received any injections for thirty-four weeks.

Using serum 6_2 as the reference serum, agar diffusion tests were done in plates to compare the bands produced by eight other antisera from the second group of rabbits. All these gave two heavy bands which joined with the heavy bands produced by 6_2 (reactions of identity[6]). Extract E, therefore, contained two major antigens to which these rabbits responded with production of antibody. The presence of two, rather than a single *major* antigen, as has been described,[10] may be attributable to the difference in the manner in which the extracts were prepared. In addition to No. 6, some animals formed antibody, as judged by band formation in agar, to one or more other antigens present in the extract (see Table I).

Relationship of the Two Major Antigens in Extract E to the Component Responsible for Its Reactivity in the Skin of Dust-Sensitive Persons.—In the following, we proceeded on the assumption that if either of the major antigenic components were responsible for the reactivity of extract E in the skin of dust-sensitive subjects, then the reactive component should go down with the immunologic precipitate. Accordingly, a mixture of 10 ml. of serum No. 14_2 and 10 ml. of extract E 1:40 was made, judged by flocculation tests to represent optimal proportions. After this had stood for three days at 4° to 6° C., it was centrifuged and the supernatant was removed. The tube was wiped out with sterile gauze and the precipitate was washed once with cold sterile saline. Dissociation of this precipitate was attempted by mixing it with 12 ml. of 15 per cent sodium chloride solution.[7] After this mixture had stood for

eighteen to twenty-four hours at 35° C., it was centrifuged and the supernatant was removed. No precipitate formed when acetone was added to this supernatant to a concentration of 33 per cent. When the amount of acetone was increased to 66 per cent to precipitate any skin-reactive material present, the mixture became slightly opalescent. This precipitate was separated in the centrifuge, saline was added to it, and the insoluble material (rabbit serum protein, etc.) was separated in the centrifuge. The supernatant was inactive in the skin of a suitable dust-sensitive subject. The supernatant of the original mixture described previously, which formed no bands in agar when tested with antiserum, was tested in the skin of the same subject before and after removal of the rabbit serum proteins by heating to 90° C. In confirmation of the findings of Boatner and Efron[8] and Rimington, Stillwell, and Maunsell,[9] heating the dust extract under study did not diminish its reactivity in the skin. When diluted to give a final concentration of extract E of 1:40,000, the heated and unheated supernatants reacted to the same degree in the skin as extract E freshly diluted to 1:40,000.

Because of the complex nature of the serologic system, one could not be certain that the mixture of antiserum and antigen had been made in optimal proportions or, indeed, under circumstances wherein a substantial proportion of the antigen in question had been removed. The same type of experiment was carried out, therefore, in the region of clear-cut antibody excess. Mixtures containing equal parts of antiserum and extract E 1:400 were prepared with three different rabbit antisera. After five days at 4° to 6° C., the precipitate present was separated in the centrifuge and the supernatant was saved for testing. In agar diffusion tests the unheated supernatant formed the same number of bands with extract E as the corresponding antisera diluted 1:2, showing that precipitating antibody was in excess in these mixtures. A portion of each supernatant was heated as described previously. Both the heated supernatants, diluted so as to bring the extract to 1:40,000, and extract E, diluted 1:40,000, were equally reactive in the skin of a dust-sensitive subject. The same result was obtained when the experiment was repeated using extract E 1:4,000 instead of 1:400.

These results indicate that the skin-reactive material was not precipitated by the antiserum. That precipitation was complete, or nearly complete, in the first experiment done with equal parts of antiserum and extract E 1:40 was indicated by the failure of the unheated supernatant to form bands in agar diffusion tests when tested with antiserum. In the experiments done with the extract diluted 1:400, the formation of bands indicated a definite excess of precipitating antibody. It was concluded from this experiment, therefore, that the skin-reactive component of extract E was not identical with either of the major precipitating antigens in the extract. This indicates that the *in vitro* standardization of house dust extract by the agar diffusion technique, as suggested by Wodehouse,[10] will be unwarranted unless a correlation between the specifically precipitable antigens and the skin-reactive component of the extract is first established.

To test further the effect of heat, extract E diluted 1:40 was heated for twenty minutes at 60° and 80° C. and each heated sample, together with an unheated control, was tested by agar diffusion in plates. Heating to 60° C. was without effect, but the sample heated to 80° C. formed no bands.

It is evident that the serologic activity of extract E was more easily destroyed by heating than the allergenic activity, since the latter was apparently unchanged after heating for ten minutes at 90° C. Rimington and his associates[9] reported that the allergenic activity of their purified dust extract was undiminished after thirty minutes of refluxing with 0.5N hydrochloric acid at 100° C.

Tests for complement fixation were done with the heated supernatant from the first mixture made with extract E 1:40 and antiserum 14_2. It was readily shown that heating extract E alone enhanced its anticomplementary activity more than fivefold. (For this reason, fixation tests with the heated extract were not attempted.) However, the heated supernatant from the immune precipitate was, roughly, five times *less* anticomplementary than unheated extract E. This indicates that one, or both, of the major antigenic components of the dust extract was the chief source of its anticomplementary activity. Six antisera, including 14_2, were tested for fixing capacity with this supernatant. There were varying degrees of fixation by the sera, one fixing completely with dilutions of 1:800 to 1:6,400; 14_2 fixed, but not completely, with dilutions of 1:800 and 1:1,600.

It appears, therefore, that removing the immune precipitate and heating the supernatant to 90° C. abolished the capacity of the dust antigen to form bands in agar, lowered the minimal fixing dilution, and markedly decreased anticomplementary activity as compared with extract E heated in the same manner, but did not remove all fixing antigen and left its skin reactivity unimpaired. These findings, coupled with the presence of complement-fixing activity in two normal rabbit sera lacking precipitins, suggest that an antigen was present in extract E which fixed complement and which was not one of the major precipitating antigens. This conclusion cannot be other than tentative in view of quantitative considerations.

Tests With Dust Extract C.—Dust extract C was studied in complement fixation tests, by agar diffusion, and in the skin of dust-sensitive subjects. In agar diffusion, two faint bands were produced in tubes which appeared to be different in character from any of those produced by the homologous extract E; in plates, a single hazy band was produced, which did not join with the two major bands produced by extract E. In complement fixation tests, seven of the nine antisera tested fixed completely with dilutions of extract C ranging from 1:2,000 to 1:32,000. With only two of these seven sera, however, was the minimal fixing dilution of extract C as high as that of extract E, whereas in the other five sera this was two to sixteen times lower. It appears, therefore, that the antigenically reactive substances in extract E had not been concentrated in the course of the preparation and "purification" of extract C. Fur-

thermore, it was distinctly less active than extract E in skin tests. We believe, therefore, that extract C was composed chiefly of relatively inert material.

Tests With Other Extracts.—A concentrated fraction,* which was prepared from freshly ginned cotton in the same manner as that of extract E, fixed complement in low dilution only, produced two very faint bands in agar, and was not reactive in the skin of dust-sensitive subjects. This finding is not surprising in view of the conclusions of others[11, 12] that aging is required for the development of allergenic activity in cotton and other similar materials.

An extract prepared from dust collected in a large library, and concentrated so that 1 ml. was equivalent to 1 gram of dust, formed bands in agar diffusion tests which were almost as heavy as those formed by extract E, but in skin tests a dilution of 1:30 was approximately equivalent to that of extract E diluted 1:40,000. Here again, allergenicity is not correlated with band formation in agar. A second extract was prepared from dust collected with an electrostatic precipitator in the home of a clinically dust-sensitive asthmatic patient. Two grams was thoroughly extracted by mixing with 30 ml. of water in a Waring Blendor. This extract formed two faint bands in agar diffusion tests and it was reactive in the skin of a dust-sensitive subject in a dilution of 1:2.

One of the major serologically active components of extract E and skin-reactive activity were present in simple aqueous extracts of old cotton mattress stuffing prepared by us.

Tests With Components of the Cotton Plant (Gossypium barbadense).—The possibility that antigens from either the cottonseed or the plant itself might be the source of some of the antigenic components of extract E was also investigated. Extracts of the finely ground seed prepared with Coca's extracting fluid did not react in interfacial tests with five immune sera, but an extract prepared with physiologic saline reacted markedly, not only with these sera but also with three normal rabbit sera. The difference in the behavior of these two extracts perhaps may be attributable to the difference in pH during extraction. Dried fresh leaves and stems of the cotton plant, extracted with physiologic saline, did not react in interfacial tests with four immune sera. These studies are still in progress.

DISCUSSION

The results reported herein indicate that a concentrated aqueous extract of old cotton contains at least five antigenic components, two of which (major antigens) were demonstrable with most of the antisera prepared in rabbits. Strong evidence is presented to indicate that these two major antigens were not responsible for the reactivity of extract E in the skin of dust-sensitive persons. As these antigens and skin reactivity were lacking or present in very small amounts in an extract prepared from freshly ginned cotton, they presumably develop in cotton with the passage of time. They presumably

*Kindly supplied by Dr. Samuel M. Gordon.

must arise from growth of organisms (bacteria, molds), from spontaneous chemical change (oxidation?) in cellulose or other components of the cotton fiber, or as an atmospheric deposit. As allergenic activity appears to be widely distributed in house dust and stuffing from upholstery and old cotton mattresses, decaying vegetation with which the ground out-of-doors is littered likewise might be regarded as a possible source of the same or very similar allergenic activity. Were this the case, however, one might expect that such allergenically active components, being water soluble, would be leached out by rain and lost.

Failure to demonstrate neutralization of the allergen in the experiments in which supernatants from mixtures of antiserum and extract E were tested for allergenic activity cannot be attributed conclusively to lack of antibody in the rabbit sera for the allergenic component. The first of the supernatants obtained from the mixture of equal volumes of serum 14_2 and extract E 1:40 required 500-fold dilution prior to carrying out the skin tests. This might have caused dissociation of a soluble antigen-antibody complex, if such were present, releasing allergenic material. Also, in the later tests, the effect of heat upon such soluble antigen-antibody complexes is not known. However, these possibilities appear to us as an unlikely explanation of the results. On the other hand, the conclusion that allergenic activity was not removed in the antigen-antibody precipitate cannot be questioned. The finding that a dust extract, skin reactive in a dilution of 1:30, formed dense bands comparable to those formed by extract E 1:40, clearly reactive in skin in a dilution of 1:40,000, is further evidence that there is no correlation between the formation of bands with rabbit antisera to extract E and allergenic activity.

It is probable that the two antigens in extract E forming the heavy bands in agar diffusion tests were the ones mainly responsible for fixation in the complement fixation tests. With many of the antisera, these bands formed very close together, which may indicate approximately equal amounts of antibody to each, and this would make it difficult to demonstrate zones of fixation. Those antigens in extract E that formed faint bands near the antigen well were probably present in such low concentrations that they would not have fixed C′ in the dilutions of extract E used.

The results of precipitin tests with extracts of cottonseed and cotton plant indicate that they were not a source of antigenic material in extract E.

SUMMARY AND CONCLUSIONS

1. A concentrated aqueous extract of old cotton (extract E) gave rise to precipitating and complement-fixing antibody in rabbits.

2. Extract E contained at least five antigenic components, two major and three minor, and at least one skin-reactive component.

3. These antigenic components were either not present or present in very small amounts in a concentrated aqueous extract of freshly ginned cotton,

which was likewise unreactive in the skin of a dust-sensitive subject; nor were they found in extracts of cottonseed or the leaves and the stems of the cotton plant.

4. Heating extract E to 90° C. enhanced its anticomplementary activity and abolished its capacity to form immunologic precipitates, but did not alter its reactivity in the skin of dust-sensitive subjects.

5. Removal of an immune precipitate from extract E in the presence of excess antibody did not alter the reactivity of the extract in the skin. This and other findings strongly indicate that the skin reactivity of extract E did not reside in the two major antigenic components.

We gratefully acknowledge the technical assistance of Mrs. Elizabeth T. Leach.

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THE EFFECTIVENESS OF THE RAGWEED ERADICATION CAMPAIGNS IN NEW YORK CITY

A 9-Year Study (1946-1954)

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SINCE 1936, the Allergy Division of The Jewish Hospital of Brooklyn has been estimating daily the pollen content of the air in the Borough of Brooklyn during pollinating seasons. When, in 1946, the National Pollen Survey Committee of the American Academy of Allergy standardized the techniques for pollen counting,[1, 2] the surveys were promptly enlarged to include not only all the boroughs of New York City but also the surrounding metropolitan area.[3] Coincidently, in 1946, the New York City Department of Health initiated "Operation Ragweed," a series of annual ragweed eradication campaigns within the city which have been continued without interruption up to the present time.

Variations in rainfall, sunshine, temperature, wind direction and velocity, and other meteorologic factors cause wide fluctuations in the seasonal pollen index of a community from year to year. Hence, a final evaluation of the effectiveness of "Operation Ragweed" was postponed until the influence of meteorologic variations on New York's pollen index could be weighed. After nine years of pollen surveys during which the counts were correlated daily with the weather data, the relative importance of the various meteorologic factors in this community has been established. On the basis of this information, an equitable evaluation of the effectiveness of "Operation Ragweed" may now be attempted.

For the first four years of "Operation Ragweed," a decline in the city's ragweed acreage was reported by the Health Department.[4] For the last five years, however, no further diminution was noted. On the basis of pollen counts no consistent decline in New York City's pollen index has been observed over the nine-year period. Under the circumstances, it is reasonable to assume that the full benefits of "Operation Ragweed" have been realized and that continuation of the same program will not materially alter the situation. The time, therefore, seems suitable for an evaluation of the benefits of this program.

The analysis presented here covers the longest and most intensive studies on record on what probably has been the most extended and systematic effort by a large city to rid itself of ragweed. Durham[5] previously reported on Chicago's three-year eradication drive on ragweed which proved to be completely

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ineffective. Durham's observations and the data provided in this analysis of New York's efforts should provide some guidance to those communities throughout the country which contemplate embarking on their own local projects for ragweed eradication.

SCOPE OF POLLEN SURVEYS

A. *In New York City.*—Throughout the ragweed season daily counts were made from greased slides exposed for twenty-four-hour periods in Durham shelters at various stations throughout the five city boroughs. Within the city there were 11 stations studied for periods varying from one to nine seasons for a total of 52 station-seasons. (Table I and Fig. 1). In addition to the above, Brooklyn counts, which had been started in 1936 using the old technique, were

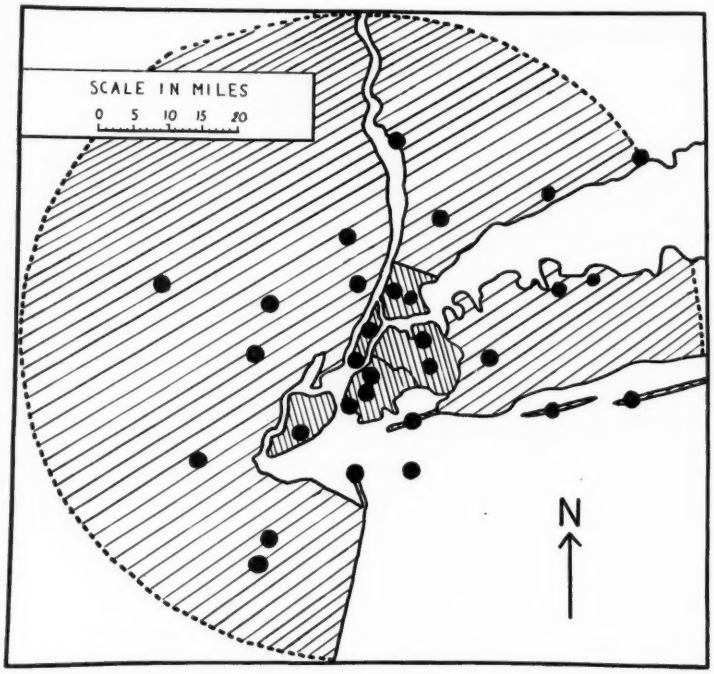

Fig. 1.—Distribution of stations studied in the New York City metropolitan area within a radius of 50 miles of Columbus Circle, 59th St., Manhattan. At the center are the five boroughs of New York City which, starting at the left and rotating clockwise, include Staten Island, Manhattan, the Bronx, Queens, and Brooklyn.

continued annually. They proved useful in comparing Brooklyn's pollen indices before and after 1946, at which point the ragweed eradication program and new counting methods were instituted.

B. *In the Metropolitan Area Around New York City.*—Outside of the city's borders, in the area within approximately a 50-mile radius of Columbus Circle in New York City, 19 stations were surveyed, providing 46 seasonal studies (Table II and Fig. 1). Included were stations in New Jersey, Connecticut, and in Westchester County and Long Island in New York State. There was, in addition, a station on the Atlantic Ocean at Ambrose Lightship.

TABLE I. STATIONS STUDIED IN NEW YORK CITY, 1946 TO 1954 INCLUSIVE

BOROUGH	STATION	1946	1947	1948	1949	1950	1951	1952	1953	1954	TOTAL STATION-SEASONS
Brooklyn	The Jewish Hospital of Brooklyn	x	x	x	x	x	x	x	x	x	9
	Brooklyn College			x							1
	Fort Hamilton				x						1
Manhattan	U.S. Weather Bureau Central Park		x	x	x	x	x				5
	U.S. Weather Bureau Battery	x		x	x	x			x	x	6
Queens	Rockaway Beach Hospital		x	x	x	x	x	x	x	x	8
	Chamber of Commerce Bldg. Flushing	x	x	x	x	x	x	x	x	x	9
	Jamaica Hospital		x								1
Bronx	Montefiore Hospital	x	x	x	x	x					5
	Fordham Hospital						x				1
Staten Island	Sea View Hospital	x	x	x	x	x	x				6
Total	11 stations										52

This analysis, therefore, covers the findings obtained with the standard technique from 1946 to 1954, inclusive, at 30 stations in and around New York City and includes data on 98 station-seasons. It also makes use of the counts made with the old technique at the Brooklyn station during the ragweed seasons of 1937 to 1954, inclusive.

TABLE II. STATIONS STUDIED AROUND NEW YORK CITY, 1946 TO 1954 INCLUSIVE

STATE	STATION	1946	1947	1948	1949	1950	1951	1952	1953	1954	TOTAL STATION-SEASONS
New Jersey	New Brunswick		x	x	x	x	x	x	x	x	8
	Verona	x	x	x	x						4
	Maplewood		x	x	x						3
	Sandy Hook			x	x						2
	Freehold			x							1
	Marlboro				x						1
	Teaneck		x								1
	Westwood			x							1
	Dover			x							1
Connecticut	Bridgeport			x	x	x	x	x	x	x	7
	Stamford									x	1
New York	Garden City		x	x	x	x					4
	Long Beach							x			1
	Fire Island		x								1
	Huntington								x		1
	Northport									x	1
	White Plains		x	x	x	x					4
	Croton	x	x								2
	Ambrose Lightship—New York Harbor				x	x					2
Total	No. of Stations 19						No. of Station-Seasons				46

TECHNICAL CONSIDERATIONS

Durham slide shelters were set up at various stations under similar exposure conditions which approached the standard as closely as possible.[1] Greased slides, prepared at and distributed from the Allergy Laboratory of

The Jewish Hospital of Brooklyn, were exposed in the shelters between 8 and 9 A.M. for twenty-four-hour periods and were returned to the laboratory in sealed containers at regular intervals. At the laboratory, these were stained and counted according to the standard technique by trained staff members and technicians.

Pollen counts were recorded in terms of the number of ragweed pollen granules per square centimeter of exposed slide per twenty-four-hour period. Multiplying this figure by the standard coefficient 3.6 (or by 1.8 for the slides exposed in the old shelter) yielded the approximate pollen content of a cubic yard of air for the twenty-four-hour period.[2] From the daily ragweed counts of all stations within the city an average reading for New York City was computed. Seasonal totals for a station were obtained by adding all of the daily pollen counts of the entire ragweed season at that station. This figure, however, is inadequate for the clinical appraisal of the community as a pollen hazard to the sensitive patient. For this purpose, a pollen index was computed for each ragweed season at each station according to Durham's formula.[6] This index is obtained by a summation of three factors computed as follows: (1) the seasonal total divided by 200; (2) the number of days on which the count exceeds 25; and (3) the highest single count of the season divided by 100. All calculations in this formula are based on counts in terms of the number of pollen granules per cubic yard of air. From the indices of the stations studied each season, an average pollen index for the entire city was computed for each year.

METEOROLOGIC STUDIES

Meteorologic data provided by the U.S. weather stations in the vicinity of the sites of survey were analyzed and correlated with the pollen data. The weather station at the Battery at the lower tip of Manhattan Island was the one nearest to the Allergy Laboratory and not only provided invaluable meteorologic information but served as a slide exposure station during six ragweed seasons (Table I). The many meteorologic factors which influence pollen counts will be the subjects of other communications.

WIND AS A FACTOR IN NEW YORK CITY'S RAGWEED PROBLEM

In previously reported surveys of Fire Island, New York,[7] and Sandy Hook, New Jersey,[8] the pronounced influence of western winds on New York City's ragweed pollen index was demonstrated. Although these stations were located on sandy beaches, bearing little or no ragweed and almost completely surrounded by the ocean (Fig. 2), they yielded high pollen counts on days when brisk westerly winds prevailed.

Most significant have been the studies made in 1949 and 1950 at Ambrose Lightship,[9] which is anchored well out in the Atlantic Ocean at the entrance to New York Harbor. The Lightship is at least nine miles from the nearest land and for purposes of comparison might be considered the center of an imaginary ragweed-free region immediately adjacent to New York City and corresponding in area and outline to the city (Fig. 2). The high pollen peak

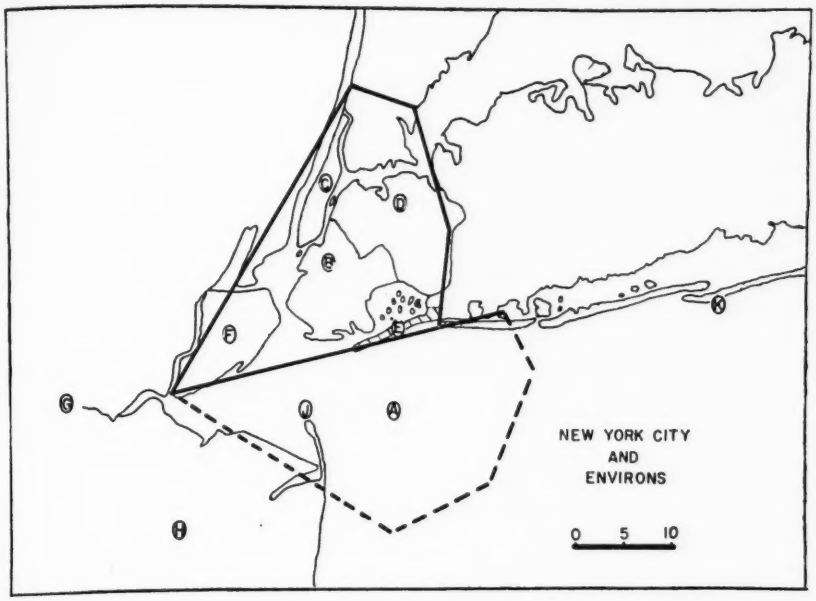

Fig. 2.—The heavily lined pentagon roughly represents the area covered by New York City. Adjacent to it, and on the ocean, a similar figure is drawn in broken outline to represent an imaginary "ragweed-free New York City." Ambrose Lightship (Station *A*) would be close to the center of this imaginary "city." Other stations on this map include (*B*) The Jewish Hospital of Brooklyn; (*C*) Central Park, Manhattan; (*D*) Flushing, Queens; (*F*) Staten Island; (*G*) New Brunswick, New Jersey; (*H*) Marlboro, New Jersey; (*J*) Sandy Hook, New Jersey; and (*K*) Fire Island, New York. (Scale is in miles.)

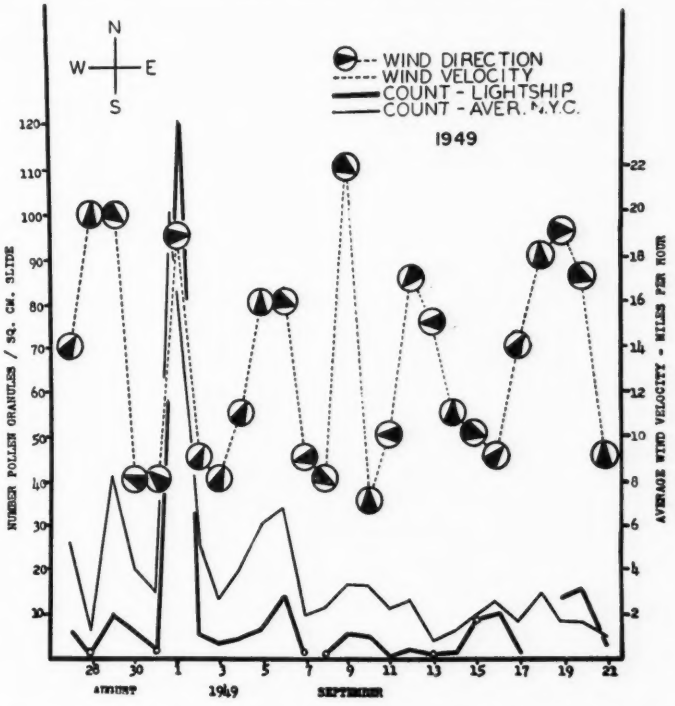

Fig. 3.—Daily pollen counts at New York City and Ambrose Lightship and their relationship to wind direction and velocity during the ragweed season of 1949.

days on the Lightship almost regularly coincided with those of the city and in some instances the counts on the ocean exceeded those of the city (Fig. 3). On these peak days, the wind almost always proved to be westerly and brisk.

On the basis of these Lightship studies, it was conservatively estimated that at least one-half of New York's ragweed pollen originated outside the borders of the city, particularly in areas to the west and southwest, and, to a minor degree, in districts to the northwest and north. Thus, theoretically at least, a perfect intramural ragweed elimination campaign could not be expected to reduce New York's pollen by more than half. The pollen surveys made during the course of the campaigns have supported this hypothesis.

"OPERATION RAGWEED"

From time to time, the Health Department of New York City has issued reports, the most recent one in January, 1955,[4] on the progress of this project. The cooperation of other city departments has been enlisted in this undertaking, the annual cost of which is estimated at $85,000. The main feature of the

TABLE III. NEW YORK CITY RAGWEED ELIMINATION CAMPAIGNS 1946-1954

YEAR	ESTIMATED ACREAGE OF RAGWEED*		MAN DAYS OF LABOR	UNUSED ACREAGE†	
	PRECEDING CAMPAIGN	ELIMINATED IN CAMPAIGN		NEW YORK CITY	BROOKLYN
1946	10,000	3,149			
1947	8,000	4,795	3,814		
1948	6,000	3,944	2,904	(Nov.) 48,300	5,900
1949	4,000	3,297	2,953		
1950	5,000	3,157	2,841		
1951	5,500	3,680	3,177		
1952	5,000	3,197	2,853		
1953	5,000	2,350	2,874	(June) 30,800 (-36%)	3,300 (-44%)
1954	4,500	3,004	2,915		

*Report of City Health Department.
†Report of City Planning Commission, November, 1948 to June, 1953.

campaign has been the spraying of ragweed with 2-4D in advance of each pollinating season. Because heavy street-flushing trucks are employed for this purpose, the weed growths which can be reached by these vehicles which traverse the city's roads have received the most intensive spraying.

In its reports, the Health Department has provided estimates on the extent of the ragweed infestation which preceded each campaign, on the acreage that was "eliminated" (sprayed) annually, and on other details of the operation (Table III). According to surveys made by the Police Department in 1946 and 1947, there were approximately 10,000 acres of ragweed in the city before "Operation Ragweed" was started.[4] All subsequent estimates were provided by the Health Department which has, for the most part, relied on the reports of the spraying teams for its information. According to these reports, the ragweed acreage has been reduced by not more than half during the nine years of campaigns. Most of this improvement was achieved during the first four years, and there has been no radical change in ragweed acreage since then. Although approximately 3,000 acres have been "eliminated" during each cam-

paign since 1950, the city total at the start of the subsequent campaign has remained more or less the same at approximately 5,000 acres. On the basis of these estimates of approximately a 50 per cent reduction in intramural ragweed acreage during this nine-year period, it is theoretically impossible for "Operation Ragweed" to achieve more than a 25 per cent reduction in the offending pollen, since approximately half of New York's airborne pollen is of extramural origin and is therefore not influenced by the city's campaigns.

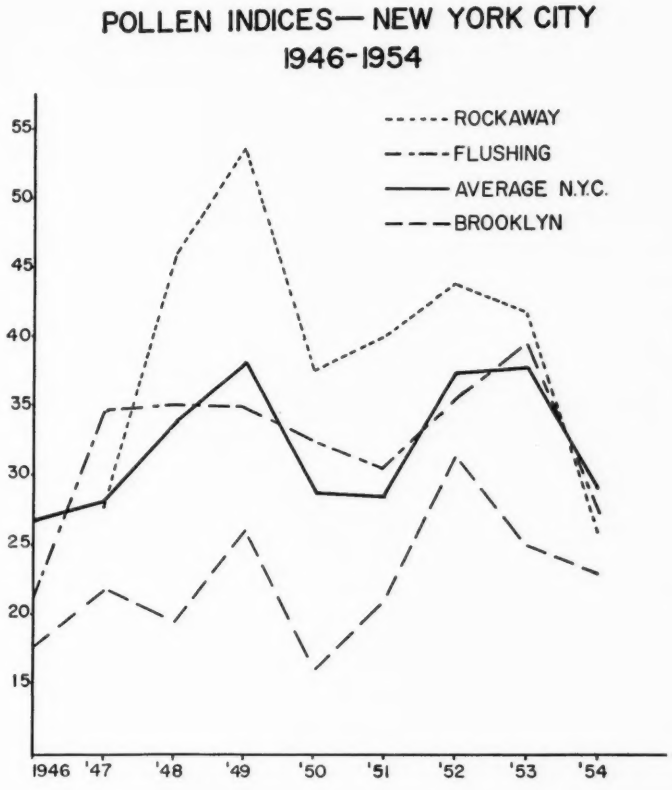

Fig. 4.

THE RESULTS OF THE POLLEN SURVEYS

A. *Effect of "Operation Ragweed" on the Pollen Indices of New York City.*— The ragweed pollen indices for New York City as a whole and for the stations at Brooklyn, Flushing, and Rockaway (Fig. 2) during the nine years of "Operation Ragweed" are graphically presented in Fig. 4. The index for the entire city has varied from 27 to 38 and has shown no consistent tendency to decline. The indices at Flushing in the Borough of Queens run close to those of the city. At Rockaway in Queens and at Brooklyn, the indices run, respectively, above and below those of the city. At none of these stations was there any indication that the indices were declining or deviating materially from the city indices or from each other. The major fluctuations shown in the

graphs may all be satisfactorily accounted for on the basis of the meteorologic conditions which prevailed during the ragweed pollinating seasons or during the months preceding them. The findings at the other city stations which were studied less extensively were in complete accord with the results obtained in the more complete surveys.

A comparison of New York City's pollen index with those of neighboring cities, where no organized efforts at ragweed elimination were made, likewise fails to provide any evidence that "Operation Ragweed" has a pronounced

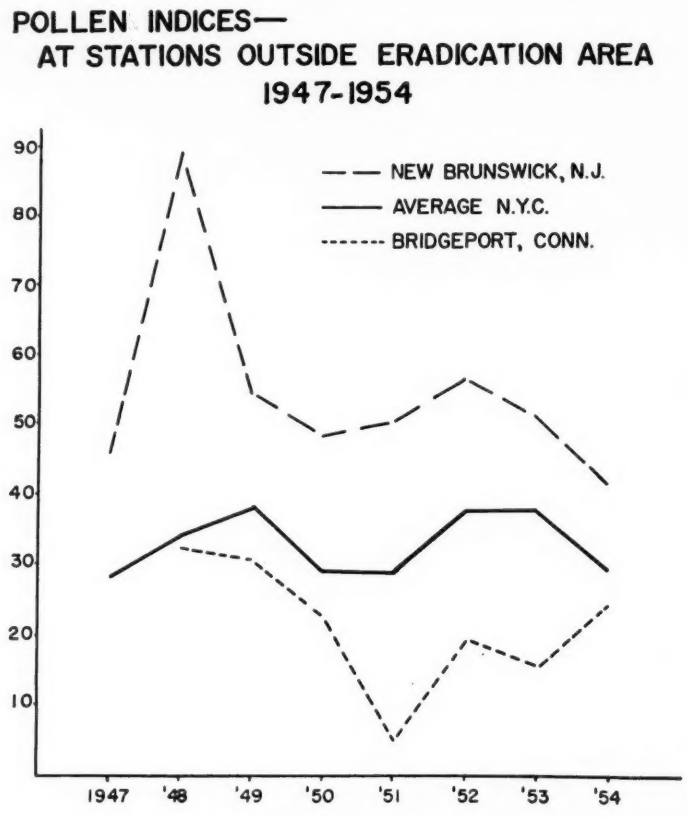

Fig. 5.

effect on the pollen index of New York (Fig. 5). At New Brunswick, New Jersey (Fig. 2), which lies about 30 miles southwest of New York in the vicinity of heavy ragweed growth, the pollen indices since 1947 have consistently been higher than those of New York. With the exception of the 1948 readings, the graphs of the two cities have run almost parallel. Since New Brunswick lies in the direction from which New York receives so much of its pollen, such a pronounced correlation of the two graphs is not remarkable. On the other hand, Bridgeport, Connecticut, which lies about 50 miles northeast of New York (Fig. 1), has a consistently lower pollen index than New York City, since it lacks dense ragweed growth of its own and receives relatively little pollen

from surrounding areas. Here again a comparison of the indices of the two cities fails to provide any indication that New York's readings have been materially reduced or noticeably affected by the eradication program.

B. *The Effect of "Operation Ragweed" on Brooklyn's Pollen Index.*—There are no available statistics by which the pollen indices of New York City as a whole, before and after 1946, can be compared. There are at hand, however, the pollen counts for Brooklyn made annually, since 1936, at the Allergy Laboratory of The Jewish Hospital of Brooklyn. Although these data represent readings at only one station and were obtained with the "old" technique of slide exposure and counting (using the 1.8 factor), they nevertheless

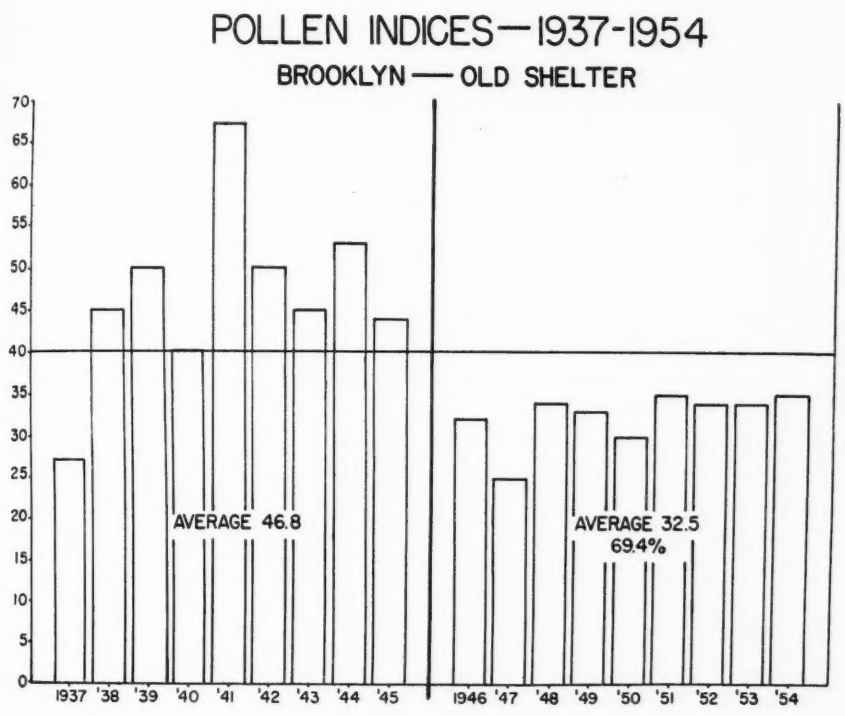

Fig. 6.—Blocks represent annual pollen indices at The Jewish Hospital of Brooklyn Station from 1937 to 1954, inclusive, based on counts made with the old technique. Heavy vertical line marks start of "Operation Ragweed." Light horizontal line represents average pollen index for Brooklyn over the eighteen-year period.

comprise the only series of uninterrupted pollen surveys which were made within New York City throughout the entire period under consideration. For this reason these data have proved to be of considerable value.

From these data, it appears that the average pollen index for this Brooklyn station, during an eighteen-year period running from 1937 to 1954, inclusive, was approximately 40 (Fig. 6). For the nine-year period preceding 1946, the average pollen index was 46.8. For the subsequent nine-year period, it was 32.5. Hence, there appeared to be a decline of 30.6 per cent in the average pollen index of this station while "Operation Ragweed" was in progress.

However, this figure needs careful interpretation since there are factors other than the eradication program which have affected the pollen index of this Brooklyn station during this period. Of these, only the two most important will be discussed at this time.

1. *The decline in vacant land:* Among the local factors affecting the pollen index of this Brooklyn station were changes in the neighborhood where the slides were exposed. Most important among these was the erection of many new buildings on what was formerly vacant weed-infected land. The building boom which affected the entire city was particularly active in the Borough of Brooklyn. In New York City, according to the report of the City Planning Commission,[10] vacant land diminished from 48,300 acres in November, 1948, to 30,800 acres in June, 1953, a decline of 36 per cent in a four and one-half year period (Table III). For the Borough of Brooklyn the decline in vacant land during the same interval was 44 per cent. Since "Operation Ragweed" involves a nine-year period which includes and doubles that covered by the City Planning Commission survey, it is reasonable to assume that the decline in Brooklyn's vacant land acreage from 1946 to 1954, inclusive, was in excess of 60 per cent. The effects of such a change upon the ragweed acreage and pollen indices of the Borough were undoubtedly great.

2. *Wind factors:* A meteorologic factor has also contributed decisively to a reduction in Brooklyn's pollen index during the last nine years. During this period, there has been an inexplicable decline in the average number of days during the ragweed pollinating season, on which the prevailing winds blew from the west or southwest at an average velocity of at least 12 miles per hour. The pronounced influence of these particular winds on New York's pollen index has been stressed.[9, 11]

For this special study the wind and pollen data for each of the 18 seasons under consideration were reviewed, the analysis being limited to the interval between August 15 and September 22. This is the period of active ragweed pollination in this area when winds may decisively influence the pollen counts. The pollen indices for the Brooklyn station were modified in this special study to cover only the shortened period under consideration.

For the entire eighteen-year period under analysis, there was an average incidence of 4.8 days per season when the prevailing winds blew from the west or southwest at an average velocity of 12 or more miles per hour (Fig. 7). For the nine-year period preceding "Operation Ragweed," this average was 5.7 days per season. For the following nine years, this average fell to 4 days per season, a decline of 30 per cent. An adequate explanation for this change is lacking, but there can be no question as to its significance in affecting the city's pollen index in recent years.

It is impossible to evaluate in percentages the degree to which these declines in vacant land and in seasonal brisk western winds have affected Brooklyn's pollen index during the last nine years. That they have contributed materially to the 30.6 per cent reduction in this Borough's index during this period is certain. They may possibly account for as much as from one- to two-

thirds of this decline. This would leave a diminution of from 10 to 20 per cent in Brooklyn's pollen index which could be attributed to "Operation Ragweed." It is reasonable to assume that these estimates for Brooklyn are fairly representative of the effects of "Operation Ragweed" as they apply to the city as a whole.

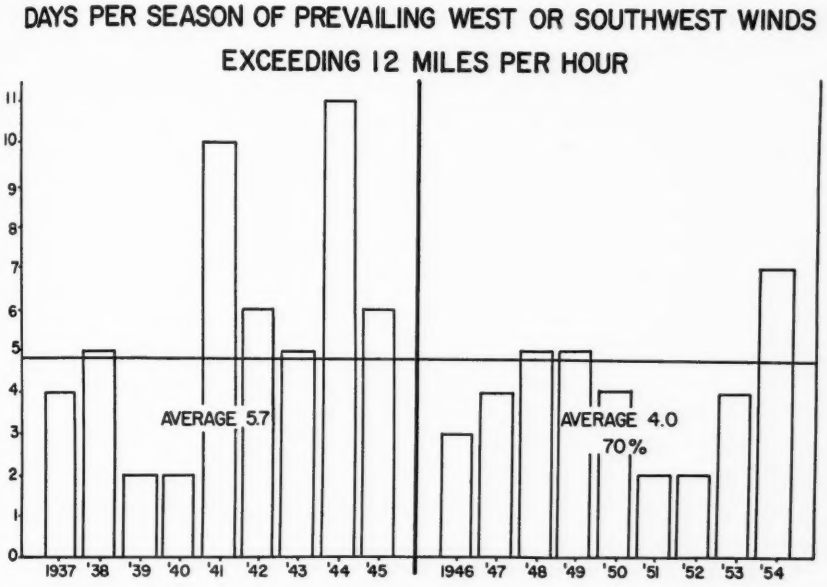

Fig. 7.—Blocks represent number of days each season when predominating winds blew from the west or southwest at velocities of 12 or more miles per hour. Light horizontal line represents the average for the eighteen-year period. Heavy vertical line marks start of "Operation Ragweed." Wind data are based on readings by the United States Weather Bureau at the Battery, at the lower end of Manhattan.

GEOGRAPHIC FACTORS INFLUENCING NEW YORK'S ELIMINATION CAMPAIGN

By all criteria, the effects of "Operation Ragweed" on the city's pollen index are not striking. Yet, limited as they are, they might have been even less apparent had there not been important geographic and meteorologic considerations which were advantageous to the campaigns.

On most sides, New York City is surrounded by large bodies of water (Fig. 2). The Atlantic Ocean and Long Island Sound form its southern and eastern boundaries except where they are interrupted by contact with Long Island. The broad Hudson River and New York Bay make up most of its western border. There are no extensive heavy ragweed concentrations to the east in neighboring Connecticut or Long Island so that eastern winds generally represent no serious problem to ragweed sufferers in New York City. Winds from the south and southeast are sea breezes and are generally free of pollen. Hence, winds blowing into the city on at least one-third of its circumference do not materially increase New York's pollen index. During the last fourteen years, these south, southeast, east, and northeast winds averaging more than 12 miles per hour in velocity constituted about 25 per cent of the brisk winds

charted in New York City during the height of the ragweed seasons. Communities which are well inland may not derive the benefit of such geographic and meteorologic advantages in their elimination campaigns.

THE OUTLOOK FOR RAGWEED ELIMINATION CAMPAIGNS

On the basis of past experience, it seems unlikely that New York City can be rendered completely free of ragweed with the present methods of eradication and by community campaigns. Moreover, it is not to be expected that the problem will be solved by legislation, no matter how authoritative. It has been pointed out repeatedly that ragweed may be totally and permanently eliminated only in those areas where, because of climatic or soil conditions, the weed is not a natural inhabitant and where contamination by wind-borne pollen is prevented by natural barriers such as broad mountain ranges, deserts, forests, or oceans.

In New York City and in most of the nonmountainous parts of New Jersey, Pennsylvania, and New York State, natural conditions favor profuse ragweed growth. The soil in these areas is contaminated with ragweed seeds which may remain viable for as long as thirty or forty years. For many years fresh breaks in the soil surface either for purposes of planting or construction will start new growths of ragweed and reinvasions of the cleaned areas.

The fact that the heavy ragweed concentrations in these states are both rural and urban renders total eradication less attainable, since it is even more difficult to wage effective campaigns in rural and partially developed areas than in cities and towns.

In the past it has been repeatedly demonstrated that anything less than complete success in the battle against ragweed is a doubtful gain. This has again been shown during the last five years of "Operation Ragweed" in New York City where, in incompletely cleared areas, the weeds have returned almost as rapidly as they were "eliminated."

To date, the results of local eradication campaigns in large cities, such as Chicago and New York, have not been encouraging. Whether it is feasible or even possible to solve the ragweed problem on a national or continental scale is a moot question. Without the participation of Canada and Mexico in such an enterprise, the prospect for ultimate success would be dim. The task would be Herculean. The cost would be staggering. The entire problem of ragweed elimination is obviously a formidable one which is not to be solved by a casual approach on a community level.

SUMMARY

1. Approximately half of New York City's ragweed pollen is generated intramurally. The remainder is brought in by brisk western winds from New Jersey, Pennsylvania, and to a lesser degree from upper New York State. Hence, the total eradication of the ragweed within the city can at best reduce its atmospheric contamination with ragweed pollen by one-half.

2. On the basis of the reports of New York City's Health Department, indicating approximately a 50 per cent reduction in the city's ragweed acreage

after nine years of "Operation Ragweed," it may be estimated that the city's ragweed pollen production may have been cut by one-half, thus reducing the pollen contaminating the air of the city by one-fourth.

3. During the nine years of "Operation Ragweed," pollen surveys were made under standard conditions at 30 stations in and around New York City, covering a total of 98 station-seasons. The results of these surveys revealed no definite tendency for the pollen index of New York City as a whole, or of any city station, to decline during this period.

4. Comparisons of New York's pollen indices during this period with those of nearby New Brunswick, New Jersey, and Bridgeport, Connecticut, where there have been no elimination campaigns, afford no indication that the course of New York's index in recent years is deviating from that of its neighbors.

5. Pollen counts at the Brooklyn station made with the old counting technique over a period of eighteen years (1937-1954) reveal a 30.6 per cent decline in the average pollen index at this station during the nine-year period (1946-1954) while "Operation Ragweed" was in progress. During this same interval, however, there was also a pronounced decline in Brooklyn's vacant land, probably in excess of 60 per cent. Coincidently, there was, in addition, an inexplicable decrease of 30 per cent in the average incidence of stiff western winds during the active pollinating seasons when such winds contribute heavily to New York's ragweed counts. In the light of these important considerations, it may be estimated that only from one- to two-thirds, or from 10 to 20 per cent, of the 30.6 per cent decline in Brooklyn's index may be attributed to the effects of "Operation Ragweed."

6. The results obtained in Brooklyn are probably representative of those achived for the city as a whole.

7. New York is a coastal city and easterly and southerly winds blowing into the city on approximately one-third of its circumference are comparatively pollen-free. Inland communities would not benefit in this manner.

8. Since ragweed grows naturally and profusely in the New York Metropolitan Area, there is little likelihood that eradication campaigns, *carried out along the lines of those employed at the present time*, will succeed either in eliminating the weed completely and permanently from the city or in decisively reducing its pollen index.

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STUDIES ON THE NATURE OF ALLERGENIC MATERIAL IN ANIMAL DANDERS AND POLLENS

A Preliminary Report

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ONE of the greatest obstacles to progress in allergy is our limited understanding of the nature of antigens. Inherent in this problem is the inadequate measure of composition and uniformity. Recent developments in physicochemical techniques now permit a more reliable assessment of these questions. It has been our purpose, by utilizing such methods as the ultracentrifuge and electrophoresis on filter paper, to pursue this type of investigation and to submit this preliminary report. We have fractionated four selected antigens and have attempted to relate these fractions to clinical sensitivity in an effort to accomplish further resolution of basic questions.

The recent literature reflects the trend toward employment of new techniques of analysis. Abramson and his co-workers[1, 2] established the place of electrophoretic and centrifugal techniques in the field of allergy. The studies of Spies and those of Coulson[3, 5, 6] on the chemical nature of cottonseed and castor bean pointed to the necessity of continued chemical studies of antigens. Friedmann[4] amplified this subject and stressed the relationship to standardization. Loveless[7] made significant contributions to the understanding of antigen fractionation using the Nielson-Kirkwood electrophoresis-convection apparatus. Dankner and his associates[8] and Perlman,[9] working separately, evolved paper chromatographic techniques for fractionation into more basic units of pollen antigens. Bookman and Wax[10] utilized ion exchange resin to fractionate ragweed. Squire[11] investigated the relationships between horse dander and horse serum by free boundary electrophoresis. Becker[12, 13] showed the presence of at least five antigens in ragweed pollen extracts, using agar diffusion technique. Stull and his co-workers[14] were able to fractionate ragweed pollen on the basis of differing water solubility constants. Scherago, Berkowitz, and Reitman[15] studied molecular size of antigens and were unable to relate this to clinical activity. Rimington and Maunsel[16] found evidence for the polypeptide-carbohydrate nature of house dust antigen in their basic chemical studies, while Campbell, Silver, and Vannier[17] utilized paper electrophoresis and chromatography in their studies with dust extracts. Stevens and his associates[18] fractionated ragweed, utilizing the free boundary electrophoresis apparatus. These investigations serve to point out the recent emphasis upon a more fundamental approach to the study of allergenic substances, using newer physicochemical methods. Each represents a step forward in our comprehension of the nature of the materials used in clinical allergy.

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MATERIALS

Four antigens were used in the experiments to be described. Two of these antigens, horse dander and cattle dander, are environmental types and two, *Cynodon dactylon* (Bermuda grass) and *Ambrosia psilostachya* (Western ragweed), represent important pollens in the Southern California area. The environmental material was procured by vacuuming groups of animals, using a clean, new bag for each species. The powdery epidermal material obtained was mixed in Coca's solution[19] and frozen and thawed several times to fragment any intact cellular substance. The solution obtained after simple filtration was precipitated in the cold in 70 per cent acetone according to known techniques.[20] The resulting precipitate was dried in vacuo, used in these experiments on a weight by volume basis, and referred to as pure extract. The unprecipitated but clarified extracts served as controls and were referred to as raw dander. The pollen was collected, cleaned by fine-screen sifting, and extracted by grinding 2 grams in 25 ml. of saline in a glass homogenizer. The final extract, after clarification by centrifugation and filtration, was used as concentrated pollen antigen.

METHODS

Nitrogen.—Nitrogen determinations were performed by direct nesslerization on each solution and on each fraction obtained. No attempt was made to differentiate protein nitrogen from nonprotein nitrogen in this study, since it was felt that this would contribute no additional information as to the degree of antigenicity of each fraction.[6, 10]

Sedimentation.—All sedimentation data were obtained through the use of a Spinco Model L analytical ultracentrifuge. The antigens under observation were suspended in a 0.2 molar solution of sodium chloride, which was found to give optimal results. Under the conditions of these experiments, it was not possible to obtain a Schlieren pattern of the pollen suspensions because of their low molecular weight. This is in accord with previous investigations.[18, 21] As a result, no sedimentation data are reported for these.

Electrophoretic Fractionation.—Our apparatus was essentially that described by Kunkel[22] for filter paper work, with minor modifications dictated by experience. A Spinco-Durrum unit[23] was also used on several occasions, but none of the patterns derived are used in this report. After a run, the paper strips were stained and read in a continuous scanning device developed in this laboratory, the details of which will be reported elsewhere.[24] Essentially, this consisted of a Beckman Model B spectrophotometer, in which a specially designed unit continuously scanned a series of paper strips and inscribed the changing optical density on an Esterline-Angus strip chart recorder. In a typical experiment, Whatman #3MM paper was used. After the paper had been allowed to equilibrate with the buffer solution, 50 Lambda of allergen, representing 0.5 mg. of nitrogen, was placed at the starting point of each of two strips, which were then superimposed. The buffer solution was Veronal

at pH 8.6, 0.1 ionic strength. It was found, after repeated attempts, that a run of six hours at 200 volts and 12 milliamperes produced satisfactory separation. All runs were made using the double thickness of paper described. One layer was saved for clinical trial, while the other was dried and stained for reading. The two epidermal materials were stained with Brom-Phenol-Blue as described by Block, Durrum, and Zweig.[23] The pollen strips were dried and sprayed with 1 per cent ninhydrin in water-saturated butanol to which pyridine was added to 5 per cent of the final volume. This method was used since it was not possible to prevent spontaneous elution of the pollen material from the paper, despite attempts to fix the antigen by various means. The treated pollen strips were dried in an oven at 100° C. for ten minutes to develop color. It was found that the color was further intensified and stabilized after remaining in total darkness for twenty-four hours. The strips to be used for skin testing were dried and cut into half-inch (12.7 mm.) segments. Drying did not affect results, yet simplified manipulation. Each segment was eluted overnight in the cold in 2 ml. of saline solution. A positive control for each antigen, consisting of routine test material, was eluted from filter paper but not otherwise treated. All eluates were placed in capped vials under sterile conditions and intradermal testing was performed on skin-sensitive persons. All samples were checked for sterility by bacteriologic culture in peptone broth prior to testing. The test samples were coded so as not to prejudice the results. The skin reactions were graded from 0 to a maximum of 4, which represented a wheal at least 1 cm. in diameter. Only Grade 3 and 4 reactions were accepted as valid, since the electrolyte buffer could conceivably induce small wheals as the result of nonspecific irritation.

RESULTS

Epidermal Antigens.—Both the cow and horse dander antigens, raw or acetone-precipitated, yielded an intensely colored dark brown solution when dissolved in saline. This color could not be removed by dialysis against saline for forty-eight hours. The color seemed to be related to protein components in that, after precipitation, the supernate became clear. Unfortunately, this color interfered with the transmission of light through the optical system of the analytical ultracentrifuge unless the sample was greatly diluted. As a result, it is possible that some components were present in concentrations too small to be resolved as peaks in the Schlieren patterns. Contrariwise, this same color intensity enabled us to follow the progress of migration on the filter paper strips in electrophoretic studies for the greater part of the run. At the end of the run, the color would become diffused due to binding, etc. This colored component, however. did not necessarily correspond to the area of the paper electrophoretic strips which would take up the Brom-Phenol-Blue stain, nor did it correspond to areas which gave the greatest skin test activity. Actually, our observations tended to associate those components in the strip immediately preceding the naturally colored areas with maximum response in skin test trials.

Another interesting general observation noted in the results of nitrogen analysis was that the nitrogen content of the precipitated epidermal antigens was always about three times that of the raw, unprecipitated material when compared on a basis of original weight (Table I). The significance of this will be discussed later.

TABLE I

ANTIGEN PREPARATION	TOTAL NITROGEN	NUMBER OF COMPONENTS			ESTIMATED MOLECULAR WEIGHT
		ANALYTICAL ULTRA-CENTRIFUGE	PAPER ELECTRO-PHORESIS	SKIN TEST ACTIVITY	
Acetone-precipitated horse dander	1.5 mg./ml.	1	2 major	1 active	40,000
Raw extract of horse dander	0.59 mg./ml.	2 plus	Multiple diffuse		
Acetone-precipitated cattle dander	2.3 mg./ml.	1	2 major	1 active	32,000
Raw extract of cattle dander	0.83 mg./ml.	2 plus	Multiple diffuse		
Cynodon dactylon extract	0.24 mg./ml.	Diffuse	6 with ninhydrin stain	At least 3 active	Less than 10,000
Ambrosia psilostachya extract	0.33 mg./ml.	Diffuse	7 with ninhydrin stain	At least 3 active	Less than 10,000

In the analytical ultracentrifuge studies, the raw cow dander gave evidence of at least two major peaks only one of which could be demonstrated in the precipitated cow dander solution. The color density previously referred to can be seen sedimenting with the major component (Fig. 1) as a dark area occupying the right side and greater portion of the photographic film. The less intensely colored horse dander antigen shows only one clearly defined peak in both the raw and precipitated states (Fig. 1).

Through calculation of the sedimentation constants for the precipitated epidermal antigens, the average molecular weight was approximately 32,000 for cattle dander and 40,000 for horse dander. This cannot be regarded as much more than theoretical inasmuch as the calculations make assumptions as to molecular size, density, and a spherical shape which is rather unlikely in the case of large molecular weight proteins. Due to lack of a separatory cell, we were not able to obtain the individual components for skin test correlation studies.

A series of experiments were carried out using paper electrophoretic techniques. The results contained in this article cannot reflect the time and trials required before it was possible to obtain controlled behavior of the materials under study. The number of variables is considerable; these range from the type of apparatus itself to variations in current, buffer, time, etc. Various dyes were tried in order to obtain the most clear-cut definition. Even fluorescence was attempted, with interesting but not constant results. The automatic scanning device previously mentioned converted the stained strips

into curves of intensity against distance with more rapid interpretation of results. Under the conditions used, at least three major peaks were obtained with the precipitated cattle dander, while the precipitated horse dander showed two major components. Eluates of one-half inch segments of the strips were tested for skin reactivity in sensitive patients. It was found that positive skin test activity could be correlated with the eluates corresponding with the slowest moving major fraction (Figs. 2 and 3). No electrophoretic data are presented for the raw epidermal material, since patterns obtained with these were diffuse and undifferentiated.

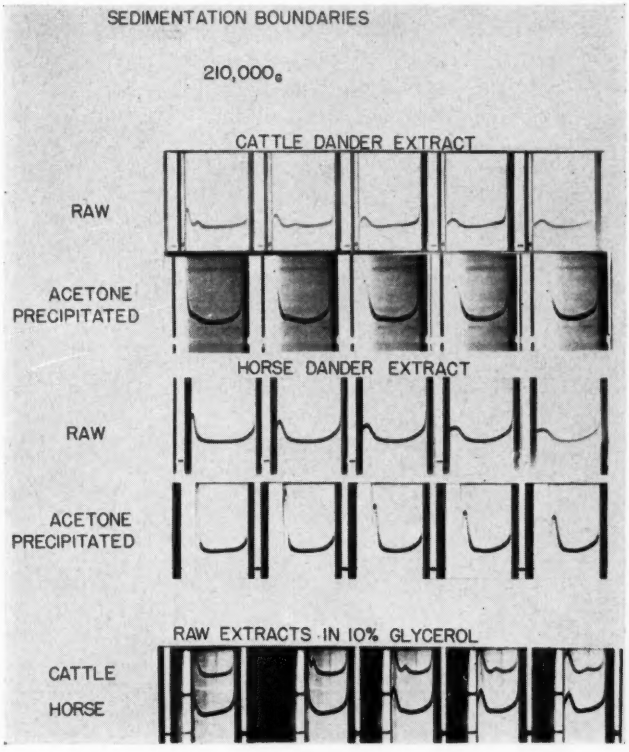

Fig. 1.

During the course of this study, the question of a possible correlation between the precipitated epidermal antigens and the whole serum of both cattle and horse concurrently arose (Figs. 2 and 3). It can be seen that the precipitated antigens tend to follow the migration rate of albumin components of the respective sera.

Pollen Antigens.—Our results indicate that pollen antigens are quite different from the epidermal type. The protein nitrogen content of the pollen extracts was found to be much lower than the nitrogen content in an equal weight of epidermal antigen. Thus, the extract of *Ambrosia psilostachya* contained 0.33 mg. nitrogen per milliliter and *Cynodon dactylon* 0.24 mg. nitrogen

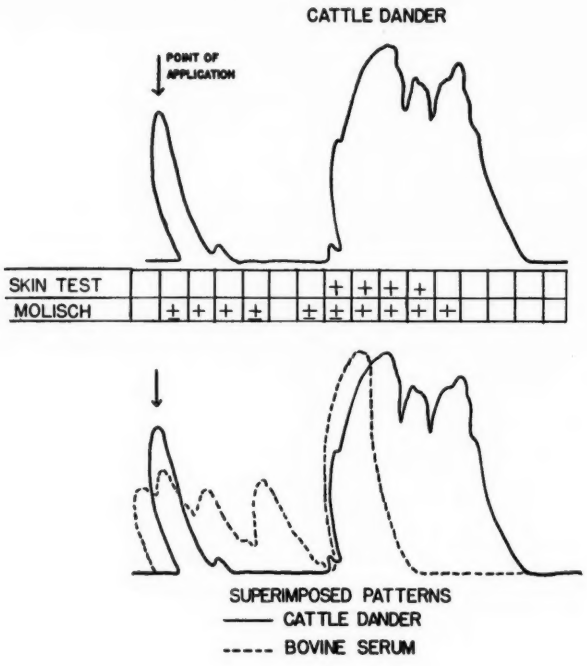
CATTLE DANDER
POINT OF APPLICATION
SKIN TEST
MOLISCH
SUPERIMPOSED PATTERNS
CATTLE DANDER
BOVINE SERUM

Fig. 2.

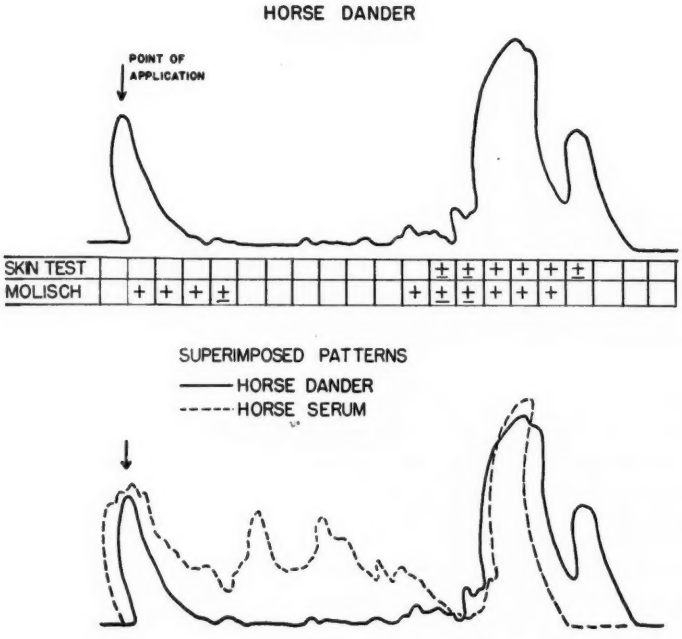
HORSE DANDER
POINT OF APPLICATION
SKIN TEST
MOLISCH
SUPERIMPOSED PATTERNS
HORSE DANDER
HORSE SERUM

Fig. 3.

per milliliter of solution as compared with 1.5 to 2 mg. nitrogen per milliliter in the epidermal antigen extracts. These and subsequent studies were carried out on undialyzed material, since we were interested in the whole substance and its behavior upon paper electrophoresis.

Using paper electrophoretic techniques, we obtained six components with extracts of *Ambrosia psilostachya* and seven components with extracts of *Cynodon dactylon*. Several of the more slowly migrating components which remained near the original point of application showed similar rates of migration in the case of both pollens. This similarity was not reflected in antigenicity of the eluted fractions. On skin testing of the fractions, however, it was found that no single electrophoretic component was clearly implicated

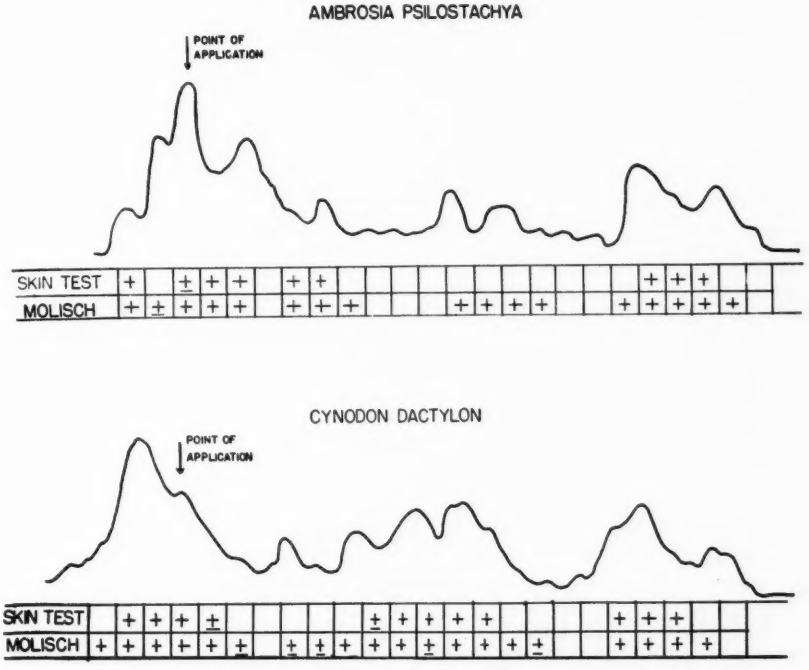

Fig. 4.

as a location of major antigenicity. Usually, two or more strip segments showed skin test activity (Fig. 4). Since considerable attention has been focused upon the existence of polysaccharide complexes in these materials, we routinely performed a Molisch test on all eluates. Generally, strongly positive Molisch reactions occurred in material which showed skin test activity or which showed as stained components on the paper strip. Significant reactions did not occur in either antigenically inactive or unstained areas of the strips. It should be noted that such tests were similarly positive when applied to the active areas of epidermal antigen paper strips.

DISCUSSION

The experimental procedures and the results we have described permit us to form some definite, although limited, opinions regarding the character of the several antigens which were studied. In the case of the epidermal antigens, the purification by precipitation resulted in more homogeneous material. These purified substances appear to be protein in nature with a molecular weight and an electrophoretic migration suggesting that they resemble serum albumin. Comparisons of the electrophoretic fractions with skin tests show that activity resides primarily in one component. However, presence of other major but relatively inactive components in the purified extracts points out the failure of complete isolation of a specific skin-reactive fraction. As noted in our results using the ultracentrifuge, it was necessary to dilute the material in solution in order to reduce the optical density. This procedure may have diluted certain components beyond the resolving power of the Schlieren optics. While we do not believe that this has resulted in the loss of significant fractions of the antigen, the possibility does exist. The threefold increase in nitrogen concentration in the purified epidermal extracts is more apparent than real, since the raw material contained much insoluble matter, such as hair, dirt, etc., which probably contained relatively little nitrogen. The main skin-reactive fractions in the purified epidermal extracts represent only a portion of the total nitrogen content, as can be deduced from examination of the electrophoretic curves, since other main nitrogenous components were not skin reactive. This raises the question of the value of nitrogen as a standard of potency without specific reference to procedures and interpretation.

We are satisfied that filter paper electrophoresis is a valuable tool in this type of study. However, certain features of the final patterns require explanation. It should be noted that all the curves presented are not a truly linear representation of the stained strips. This is due to the mechanical nature of the recorder in which the pen writer describes an arc instead of a straight line. Thus, there is a displacement of the upper portions of the curves to the left. The initial peak at the point of application represents pigmented, insoluble material which was clinically inactive in all instances. This fraction, therefore, may be regarded as an artifact. Also, it is necessary to realize that the patterns we have obtained were produced under highly specific circumstances which gave most clear-cut results in our hands. The electrophoretic patterns of all antigens studied could be reproduced quite consistently. In view of the relatively simple procedure involved, it suggests a better approach to standardization. The same material should yield the same pattern under standardized conditions in the hands of different workers.

Our studies with pollen antigens were limited to filter paper electrophoresis primarily. The value of the ultracentrifuge in this instance was unfortunately limited to more easily resolved substances which have molecular weights of the order of 10,000 or more. On electrophoretic separation, the pollens characteristically possessed more fractions than the epidermal antigens. This is a quite logical result of the use of crude pollen extracts, whereas the epidermal

antigens were subjected to purification procedures. There appeared to be no component specificity in that positive skin tests could be elicited with several fractions. This would support the validity of using crude pollen extracts clinically with our present limited knowledge of their composition. These observations suggest that there are inherent pitfalls in applying purification procedures to pollen extracts. Alteration or loss of the multiplicity of active components can occur; yet this loss may conceivably be masked by the remaining active fractions.

As a supplement to these observations, we have begun an investigation of the role of polysaccharides as factors in antigenicity. The stimulus for these supplementary studies is derived from the observation that skin test activity was as intimately related to carbohydrate content of electrophoretic fractions as it was to protein.

SUMMARY AND CONCLUSIONS

1. Two epidermal antigens and two pollen antigens in common clinical use were studied with particular emphasis upon ultracentrifuge and filter paper electrophoresis.

2. Fractionation of the epidermal antigens was achieved by ultracentrifuge, but the low molecular weight of the pollen antigen did not permit fractionation by this method. The molecular weight of horse dander antigen was estimated to be approximately 40,000 and that of cattle dander antigen 32,000. Molecular weights for the pollen antigens were found to be less than 10,000.

3. Electrophoretic separation was accomplished with all four antigens, the epidermal type showing fewer components than the pollen type. In relating skin sensitivity to electrophoretic components, activity appeared to be concentrated in a rather narrow area in the epidermal antigens, whereas the activity of the pollen antigens was more widely distributed.

4. The significance of these findings is discussed.

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SOME OBSERVATIONS ON THE PREVENTION OF BRONCHIAL REACTIONS TO AEROSOLS OF SPECIFIC ANTIGENS BY PRETREATMENT WITH AEROSOLS OF ANTIHISTAMINE AND SYMPATHOMIMETIC DRUGS

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PRELIMINARY studies[1] showed that aerosols of Pyribenzamine and Isuprel could prevent or ameliorate specific reactions to aerosols of haptens. Herxheimer[2] studied the role of aerosols of several drugs in the amelioration of specific reactions to antigen aerosols. He used statistical alterations in the "preconvulsion time" as a measure of protection.

This is a report of the effects of aerosols of several arbitrarily selected antihistamine and sympathomimetic drugs, alone and in combinations, on bronchial reactions to aerosols of specific antigens.

The methods used here have been described in detail elsewhere.[1] Briefly, guinea pigs were sensitized, actively or passively, to the specific antigens. They were exposed first to aerosols of the various drugs, then promptly to aerosols of the specific antigens. After the effects of the drugs had worn off, a day or two later, the animals were re-exposed to the specific aerosol. They were considered to be *protected* by the drug aerosol when they *did not* react to the postdrug antigen aerosol, but *did* react to the antigen later. Reactions were considered to be *ameliorated* when the response to the postdrug antigen aerosol was unequivocally slower in onset and weaker than the later reactions.

The effects of the following drugs were studied: Pyribenzamine, Chlor-Trimeton, Phenergan, Benadryl, Ambodryl, epinephrine, ephedrine, Neo-Synephrine, Isuprel, Tyzine, Chlor-Trimeton plus Isuprel, Pyribenzamine plus Neo-Synephrine, Phenergan plus ephedrine, Pontocaine and cocaine.

Guinea pigs were sensitized actively or passively to rabbit serum, acacia, egg white, or type VI or type VIII pneumococcal polysaccharides, at random. Since the type of sensitization and of specific antigen or hapten did not seem to influence the results, they will receive little emphasis. Passively sensitized pigs were usually challenged the day after sensitization. They remain sensitive to aerosols for one week or longer if not desensitized specifically.

Other details of the experiments are described later.

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*Massengill Student Research Fellow, 1954.

Aerosols of individual drugs were studied first.

Table I summarizes the attempts to protect 146 guinea pigs with aerosols of the five sympathomimetics and the five antihistamines listed previously. Sixty of the animals were sensitized actively, the rest passively.

TABLE I. RESULTS OF PROTECTION EXPERIMENTS WITH AEROSOLS OF SEVERAL DRUGS

NO. OF GUINEA PIGS	DRUG AEROSOL		RESULT*		
	CONCENTRATION (PER CENT)	DURATION (MINUTES)	PROTECTED	DIMINISHED	NO EFFECT
		Sympathomimetics			
		ISUPREL			
12	0.5	15	6	5	1
2	0.25	30	0	0	2
		NEO-SYNEPHRINE			
8	1.0	20	5	0	3
10	0.5	15-20	1	1	8
		EPINEPHRINE			
14	0.5	15	4	1	9
		EPHEDRINE			
6	3.0	15-20	0	0	6
19	0.5	15-20	1	0	18
		TYZINE			
6	0.2	20	0	0	6
2	0.1	20	0	0	2
		Antihistamines			
		PYRIBENZAMINE			
16	2.0	15-20	1	2	13
		CHLOR-TRIMETON			
13	2.0	15-20	4	2	7
4	1.0	30	0	2	2
		PHENERGAN			
8	2.0	10-15	0	0	8
3	2.0	30	0	0	3
3	2.0	5	0	0	3
		BENADRYL			
14	1.0	15-20	0	1	13
		AMBODRYL			
6	0.5	15-20	0	0	6
146			22	14	110

*For interpretation of results, see text and footnote to Table II.

The experiments in Table I were performed in the same detail as those shown in Table II, but for simplicity the table shows only the concentrations and durations of the drug aerosols and the results of the postdrug antigen aerosol challenges.

Isuprel was the most effective. Eleven of fourteen animals were protected, or the reactions were ameliorated, in agreement with the preliminary report.[1] Neo-Synephrine, epinephrine, and Chlor-Trimeton protected or ameliorated less than one-half of the animals. Pyribenzamine was effective in less than one-fifth of the animals. Pyribenzamine was less effective than in the preliminary experiments.[1]

TABLE II. ATTEMPTS TO PROTECT AGAINST AEROSOLS OF SPECIFIC ANTIGENS WITH PRIOR AEROSOLS OF THREE ANTIHISTAMINE AND SYMPATHOMIMETIC DRUG MIXTURES

NO. OF GUINEA PIGS	SENSITIZING ANTISERUM ML./I.P.	CONCENTRATION OF PROTECTING AEROSOLS	EXPOSURE TIME	INTERVAL (MIN.)	CHALLENGING ANTIGEN: EGG WHITE DILUTION	EXPOSURE TIME (MIN.)	REACTION GRADE	REACTION NO. OF PIGS	INTERVAL (HOURS)	RECHALLENGE WITH EGG WHITE DILUTION	EXPOSURE TIME (MIN.)	REACTION GRADE*	REACTION NO. OF PIGS	RESULTS
							I. Chlor-Trimeton and Isuprel Mixture							
4	1.0	1% Chlor-Trimeton and 0.25% Isuprel	30	10-20	1:30	10	None†	3	16-36	1:30	2.5 4.5	2+ 3+	3 1	
							3+	1	16-36					22 Protected†
10	1.25		30	10-20	1:10	10	None†	10	16-36	1:10	1-3	2+	2	1 No effect§
												3+	8	
9	1.25		15	10-20	1:30	10+	None†	9	16-24	1:30	2.0-5.5	2+	3	
												3+	6	
							II. Pyribenzamine and Neo-Synephrine Mixture							
4	1.25	1% PBZ and 0.5% Neo-Synephrine	15	10	1:30	10	1+‡	3	36-48	1:30	1.5-4.0	A.D.	1	3 Protected†
							None†	1				3+	1	
												2+	2	5 Diminished‡
5	1.25		30	10	1:10	10	1+‡	2	16-24	1:10	2.5-5.5	3+	1	
							2+§	1				2+	4	1 No effect§
							None†	2						
							III. Ephedrine and Phenergan Mixture							
6	1.25	0.5% ephedrine and 2% Phenergan	30	13	1:10	2-5	2+§	5	16-24	1:10	3-5	2+	4	No Protection†
							1+§	1			10	1+	1	
												None	1	No Diminution‡

*1+ = Mild dyspnea, long exposure, resp. vol. +.
2+ = Moderate dyspena, longer exposure, flanks retracted.
3+ = Severe dyspnea, gasping, cyanosis.
A.D. = Anaphylactic death.
†"Protected" when they *do not* react to the first but *do* react to the second antigen challenge.
‡"Diminished" when the first postdrug reaction was slower in onset and weaker than the second.
§No effect = No differences between the postdrug and second reactions to antigen aerosols.

Phenergan, Benadryl, Ambodryl, ephedrine, and Tyzine obviously had little protecting or ameliorating effect; nor did Pontocaine or cocaine protect or ameliorate. These last two were not shown in the table, since they are neither antihistamine nor sympathomimetic drugs.

Aerosols of the drug mixtures were studied next. Table II shows details of the sensitizing doses of antiserum, concentrations of the drug mixtures, duration of the protecting aerosol exposures, interval between protecting and postdrug aerosol challenges, concentration of the challenging antigen used in the nebulizer, duration of this antigen aerosol, severity of reactions to that first challenge, number of pigs which reacted similarly in each group, interval between the first and the second exposures to antigen aerosol (after the drug had worn off), duration of the second antigen exposure, severity and number of pigs which reacted similarly to this second antigen challenge. Results for each drug mixture are summarized in the last column.

The mixtures were selected arbitrarily as follows (see Table I): Chlor-Trimeton and Isuprel were the two, in their respective categories, which provided the most protection individually. Pyribenzamine and Neo-Synephrine stood next. Phenergan and ephedrine were combined because they seemed to promote local desensitization individually (see Discussion). Other combinations were not studied.

It is obvious that, under the conditions of these experiments, the Chlor-Trimeton-Isuprel mixture was much more effective than the Pyribenzamine-Neo-Synephrine combination. It is equally obvious that the Phenergan-ephedrine mixture was grossly ineffectual. This mixture did not promote the desensitization which these two drugs seemed to have done individually.

Controls.—Twenty-four animals were used. They were treated like those in Table II except that saline aerosol was used instead of a drug. One had fatal anaphylactic shock from the initial challenge. Six did not react to either challenge. It is assumed that they had not been sensitized. Fourteen of the pigs reacted similarly to both antigen challenges, as was to be expected.

The remaining three animals had strong prompt reactions to the first challenge, but recovered. They died in anaphylactic shock after the second challenge. These are discussed later.

Fifty-eight other animals were given sensitizing doses of antiserum, protecting doses of drug aerosols, postdrug, and later antigen aerosol challenges. They were not included in the tables because they did not react to either challenge. It is assumed that they had not been sensitized. It is possible that the postdrug challenge desensitized them to the second antigen challenge. This is thought to be unlikely.

DISCUSSION

The important results recorded here are the qualitative observations that aerosols of several drugs prevented some of the anticipated reactions to antigen aerosols in the immediate postdrug period. This protection did not last until the next day. Aerosols of combinations of Chlor-Trimeton with Isuprel and of Pyribenzamine with Neo-Synephrine seemed to prevent reactions to

specific antigen aerosols more effectively than either of these drugs alone. Phenergan and ephedrine were ineffectual, alone and in combination. No effort was made to explain the observed differences between the individual drugs or their combinations.

The results reported here are in keeping with those of Herxheimer,[2] who used a more complicated method to test a similar, but not identical, list of drugs.

The question of *amelioration of anticipated reactions* to the postdrug antigen aerosols requires some comment. In these experiments amelioration was not considered to have occurred unless the postdrug reaction to an antigen was *mild and slow* in onset, in comparison with a *prompt and severe* reaction the next day. These criteria are more stringent than were those in the preliminary report.

None of the control animals exhibited these strict criteria of amelioration. The three controls which reacted strongly and promptly to the postsaline antigen challenge were removed from the aerosol quickly, and recovered. They died in anaphylactic shock when challenged with the antigen later. Their reactions to the first antigen aerosol were not thought to be ameliorated, since they reacted strongly and promptly to the initial challenge.

Amelioration is a less reliable measure of drug protection than is the absence of a reaction to the first postdrug antigen aerosol. The conclusion seems justified that a drug has a low order of effectiveness if it merely ameliorates and does not prevent any of a series of anticipated reactions to antigen aerosols.

Neither protection nor amelioration were observed in 110 of 146 pigs in Table I. This seems to be the usual response when drug aerosols are ineffectual.

Local desensitization seemed to have occurred in forty of the animals; they reacted unequivocally to the first but not to the second postdrug antigen aerosol challenge. The budget would not permit clarification of this potentially important phase of protection by aerosols of drugs. This question of specific local desensitization by antigen aerosols while under the protection of antecedent and/or concurrent drug aerosols should be studied as a separate problem. It is not unlikely that drug aerosols may become useful adjuncts to the specific aerosol treatment of hay fever and asthma.[5]

This method of protecting against inhaled antigens seems to be quite innocuous. Tyzine seemed to irritate a few pigs slightly. Otherwise, no reactions to any of the nearly 200 drug inhalations were observed.

There are many variables in experiments of this sort. For example, guinea pigs of the same weight, sensitized passively with the same amount of antiserum, may react differently in the aerosol chamber. Of three such pigs, one may die in anaphylactic shock after one minute of exposure, another may develop a moderate reaction in six minutes, and the third may have a slight or no reaction in ten minutes. Reactions to aerosols are much less uniform than reactions to intravenous antigens in similarly prepared animals. A few of the other variables are the respiratory rate and depth during the aerosol, solubility of the drugs, local absorption and retention of the inhaled drugs,

topical antiallergic effectiveness, concentration of the drug used in the aerosol, duration of the drug aerosol, the postdrug interval before the first antigen challenge, and duration of the antigen aerosol exposure.

Statistical studies would require careful control of all these variables.

It is interesting that the Isuprel results were essentially the same here as before, whereas Pyribenzamine seemed less effective, alone and in combination, than in the preliminary report.[1] A possible explanation is that the animals in the preliminary report were less sensitive than in these experiments.

This method seems well adapted to the study of the effects of topically, and probably of systemically, administered drugs in the prevention and amelioration of specific reactions to inhaled antigens or haptens. Obviously, there are many ramifications of this broad problem which these limited observations suggest but do not solve.

SUMMARY AND CONCLUSIONS

1. Aerosols of five sympathomimetic and five antihistamine drugs and three combinations of these drugs were studied for their effects on specific reactions to antigen aerosols.

2. The drugs were effective, in the concentrations employed, in preventing or ameliorating the specific reactions in the following order: Chlor-Trimeton plus Isuprel, Isuprel, Pyribenzamine plus Neo-Synephrine, Chlor-Trimeton, Neo-Synephrine, epinephrine, and Pyribenzamine.

Ephedrine, Tyzine, Benadryl, Ambodryl, Phenergan, cocaine, and Pontocaine had no, or negligible, effects.

3. The experiments were qualitative or only roughly quantitative. It is probable that, with careful attention to the many variables inherent in the method used, the effectiveness of aerosols of some of these drugs can be increased.

4. This method should be readily adaptable to the screening of topical and, probably, systemic drugs which are designed to prevent bronchial reactions to inhaled antigens.

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MAINTENANCE OF ARTHUS DESENSITIZATION

Preliminary Report

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INTRODUCTION

DESENSITIZATION in the Arthus type of allergy has been reported by Grineff,[1] Opie,[2] Culbertson,[3] Cannon and Marshall,[4] and others in actively immunized rabbits. The desensitization which they produced was transient. It seemed logical to attempt to prolong this desensitization.

This is the report of some preliminary experiments designed to confirm the hypothesis that desensitization of the Arthus phenomenon can be maintained by daily injections of specific antigen. No record of prior efforts to maintain Arthus desensitization has been found.

METHODS

Rabbits were immunized by injections of 10 mg. of antigenic ascaris polysaccharide and 5 c.c. of 1:10 egg white solution, three successive days each week, until they had strong skin reactions and high precipitin titers. Several other schedules seemed equally effective in other animals. The ascaris antigen was used because it was being studied in other experiments. It is estimated[6] that each cubic centimeter of 1:5 egg white solution contains 21.6 mg. of mixed proteins. The reasons for using egg white are discussed later.

The desensitizing dose had to be determined for each animal. The first dose was given subcutaneously, followed by three or four larger ones at intervals of one or two hours, intravenously in the first experiments and intraperitoneally later. There were no reactions when they were given this way. Skin tests and precipitin titers were done and more antigen was given daily until the skin test became negative or equivocal and the precipitin titer was markedly reduced. The total desensitizing doses varied from 15 to 55 c.c. of 1:5 egg white solution and from 110 to 1,200 mg. (11 to 120 c.c.) of ascaris polysaccharide.

Skin and precipitin titers were then allowed to return to or near predesensitization levels spontaneously, in three or four days usually. The rabbits were then desensitized a second time by giving the predetermined desensitizing dose of antigen in divided doses within a few hours. Skin tests and precipitin titers were done about two hours later. If the skin reaction was positive the

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next day, more antigen was given. If it was negative, single daily doses of antigen were given intraperitoneally in the attempt to maintain desensitization.

During maintenance of desensitization, skin tests and precipitin titers were done daily.

Daily desensitizing doses were given to seven rabbits for ten to fourteen days. The doses ranged from 10 to 35 c.c. of 1:5 egg white solution (estimated[6]

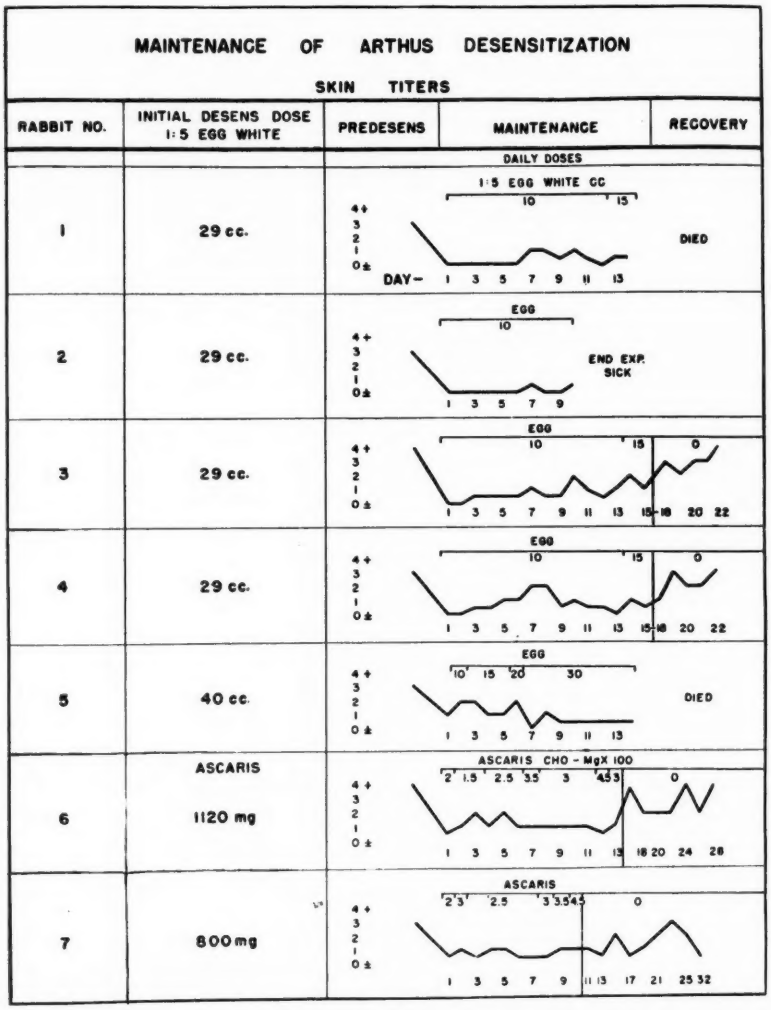

Fig. 1.—Reactions were read about twenty-four hours after intradermal injections of antigen. 4+ reactions were deep red, firm, 3 to 4 mm. thick with hemorrhagic necrotic central areas, 10 to 15 mm. in diameter. These tended to heal with scabs. The indurated red areas were usually about 2 cm. or more. 3+ reactions were similar, but with smaller and fainter hemorrhagic centers. They were less thick and firm and the diameters of the indurated red portion were usually 2 cm. or less. Scab formation was negligible. 2+ reactions showed little, if any, central hemorrhage and the total diameters of the less red and less indurated areas were 1.5 cm. or less. 1+ reactions were pink, slightly thickened, and approximately 1 cm. in diameter. Plus-minus reactions were faintly pink and barely palpable. Negative reactions had no redness or induration around the sites of the needle pricks.

at 216 to 756 mg.) in five animals and from 150 to 450 mg. (15 to 45 c.c.) of ascaris polysaccharide in the other two.

In the first four animals, an arbitrary daily dose of 10 c.c. of the egg white solution was given during most of the experiment. Fifteen cubic centimeters was given during the last few days. In the last three animals, egg white in one and ascaris in the other two, the doses of antigen were increased progressively whenever the skin reactions increased, until they became negative or only weakly positive.

Skin tests were done with 0.1 c.c. of antigen containing 1.0 mg. of ascaris or 0.5 mg. of a 1:20 solution of egg white. These test doses had been found to elicit strong reactions in sensitive rabbits.

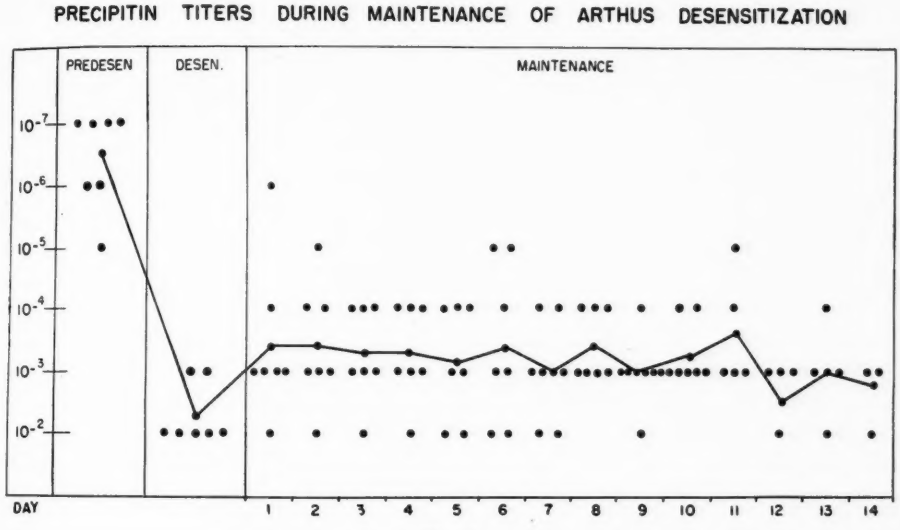

Fig. 2.

Precipitin titers were done by the ring method, using 1:3 serum and decimal antigen dilutions with saline. Readings were made after two hours at room temperature.

Controls.—Two rabbits with strong skin and precipitin titers to both ascaris and egg white were given large comparable daily doses of nonspecific antigens, acacia to one and human serum to the other. The daily skin and precipitin titers with the specific antigens were not altered appreciably.

RESULTS

Fig. 1 shows the amounts of antigen used to produce desensitization initially, the daily doses used to maintain it, and graphs of the daily skin reactions in each of the seven rabbits. Note that the initial desensitizing doses caused skin reactions to become negative in the first four, equivocal in two (Nos. 6 and 7), and weakly positive in one (No. 5) of the animals. It is obvious that a marked degree of desensitization was maintained in all of them. The skin reactions

returned to their predesensitization titers in four animals on the second (No. 6), third (No. 4), seventh (No. 3), and twelfth days (No. 7) after the antigen injections were stopped.

Fig. 2 summarizes the daily precipitin titers. The average reduction was from slightly less than 10^{-7} to slightly more than 10^{-3}. None of them fell below 10^{-2}. In the individual rabbits there was only a rough parallelism between the precipitin titers and the intensity of the skin reactions; that is, at times the precipitin titers increased slightly when the skin reactions decreased, and vice versa, and changes in one would precede or lag behind changes in the other.

DISCUSSION

These observatons confirm the working hypothesis that Arthus desensitization can be maintained, in varying degree, by daily doses of specific egg white and ascaris polysaccharide antigens. Grineff[1] reported complete desenstization with 2.0 c.c. of 1:5 egg white intravenously in two rabbits. Two control rabbits reacted strongly. He duplicated the same responses in the same rabbits twice.

Opie[2] reported complete Arthus desensitization and disappearance of precipitins with crystalline egg albumin, but not with egg white in "enormous amounts." An analysis of his data disclosed some interesting facts which previous reviewers have not mentioned: (1) He used only three rabbits, one for egg white and two for crystalline egg albumin; he did not mention having used others. (2) He used 250 mg. of crystalline egg albumin, a refined antigen, but only about 260 mg. (5.0 c.c. of 1:2) of egg white, a mixture of antigens. (3) In spite of this, the egg white reduced the skin reaction from 4+ to 1+ and the precipitin titer from 1:20,000 to 1:1,000. (4) He suggested that larger amounts of antigen mixtures might desensitize completely, but failed to test that hypothesis. These experiments (Fig. 1 and 2), using 525 to 860 mg. of egg white, indicate that he would have obtained complete desensitization had he done so.

The selection of egg white, a complex antigen, for immunization and desensitization requires some explanation. Its use seemed to be justified by the following considerations: Egg white desensitized and maintained desensitization. The premaintenance desensitization produced by egg white was complete in four rabbits and marked in the fifth rabbit. Crystalline egg albumin was less effective than egg white in the one animal (not shown) in which it was used. In two desensitizing attempts, with larger doses (440 to 485 mg.) than Opie's,[2] and in seven days of maintenance doses of 200 to 450 mg., the daily skin reactions were as weak as 1+ only once. Random samples of egg white elicited more uniform skin reactions than did three different samples of crystalline egg albumin. Vaughan and Kabat[5] showed that crystalline egg albumin was by no means a pure antigen and was inferior to egg white in eliciting Arthus skin reactions.

Maintenance of desensitization (Fig. 1) was virtually complete in rabbit No. 2, and for five to ten days in all but No. 6. The fluctuations in skin reac-

tions in Nos. 3, 4, 5, and 6 have not been explained. It is tempting to assume that the stabilization of the skin reactions at low levels in Nos. 5 and 6 was due to the increasingly larger daily doses of antigen. Support of this idea is found in two earlier observations (not shown) that skin reactions returned to predesensitization levels in two days on 10 mg. doses and in four days on 50 mg. doses of ascaris antigen. Perhaps an earlier increase in the relatively small daily doses of 10 c.c. would have had similar stabilizing effects in rabbits No. 1, No. 3, and No. 4.

Failure of precipitin titers to parallel the skin reactions is not disturbing, since it has been observed by others.[2-4] The results in Fig. 2 do indicate, however, a distinct reduction in circulating precipitins during the period of daily doses of antigen. Quantitative antibody nitrogen determinations seemed irrelevant to these simple attempts to maintain Arthus desensitization.

All the animals became thin and listless during the experiments. All had ear sloughs in areas in which antigen had not been injected and from which blood had not been taken. Two of them died on the fourteenth day of maintenance, one in shock and the other from peritonitis. Desensitization was discontinued in rabbit No. 2 after ten days because he sickened. He recovered ultimately.

This preliminary report is limited to the attempts to confirm the working hypothesis. Further studies were deferred, pending the results of current efforts to obtain synthetic homogeneous antigens and haptens.

Many interesting problems arose during these studies. Answers to them have not been found. For example: (1) What are the relative roles of antibody neutralization and of immunologic unresponsiveness to antigen in prolonged maintenance of desensitization? (2) How much total antibody can rabbits produce in response to such large doses of antigen? (3) How long can antibody production continue to neutralize large daily doses of antigen, and how efficiently will it respond to antigen increments? (4) What is the mechanism which disposes of such large amounts of antigen? Of antibody? (5) When the mechanism for antibody disposal is in "high gear," will it remain specific for the antigen, or will it dispose of coexisting antibodies which are specific for other antigens? Some observations during these experiments suggest this possibility.

SUMMARY

Arthus desensitization was maintained for ten to fifteen days, in seven rabbits, by daily injections of specific antigens. Egg white and ascaris polysaccharide were used arbitrarily.

Many interesting problems arose, but no effort was made to solve them in these introductory experiments.

Maintenance of Arthus desensitization has not been reported before.

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EFFECTS OF ADRENOCORTICOTROPIC HORMONE ON PULMONARY FUNCTION IN COR PULMONALE

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THE usefulness of adrenocorticotropic hormone as a bronchodilating agent in bronchial asthma has been demonstrated by various authors.[1-7] In patients with cor pulmonale, however, this treatment has been reported to carry therapeutic hazards causing edema of the alveolar membrane and, consequently, a decrease in the arterial oxygen saturation.[6, 8-10] Large amounts of adrenocorticotropic hormone were used in these studies.[8, 9] Small doses of adrenocorticotropic hormone, on the other hand, are therapeutically useful without causing undesirable side effects.[5, 6] It was decided, therefore, to evaluate the effect of treatment with small doses of adrenocorticotropic hormone on pulmonary function in patients with chronic lung disease and cor pulmonale, some of whom had signs of congestive heart failure before the treatment and some at the time of treatment.

MATERIAL AND METHOD

Eight studies of pulmonary function tests were performed before and after treatment in five patients, aged 35 to 65 years. All the patients suffered from cor pulmonale, confirmed by right ventricular strain in the electrocardiogram, as a result of chronic bronchitis and obstructive emphysema. Hypoxemia and decreased ventilatory function were present in all. Adrenocorticotropic hormone (ACTH*) was administered daily by intravenous drip infusion, 3 to 10 mg. in 500 c.c. 5 per cent glucose solution, or as a gel,† 20 to 40 mg. intramuscularly, for five to nine days.

Vital capacity was measured in all cases and maximum breathing capacity in all but one (Case 5). Normal values were predicted according to the criteria of Baldwin and associates.[11] Arterial blood gases were determined by the method of Van Slyke and Neill.[12]

CASE REPORTS

CASE 1.—K. M., a 35-year-old man, was admitted to the hospital because of a severe attack of bronchial asthma. He began to complain of respiratory distress three and one-half years ago. One and one-half years ago his ankles became swollen and high blood pressure, albuminuria, hematuria, and cylindruria were found. The patient was kept on a salt-poor diet and the edema disappeared.

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*Manufactured by Armour Laboratories, Chicago, Illinois.

†1 mg. = 1 I.U.

On admission, he was cyanotic, dyspneic, and orthopenic; wheezing sounds could be heard from a distance. He was sweating profusely. The temperature was normal; respiration was 30 per minute. The pulse was 120 per minute, regular; the blood pressure was 130/85 mm. Hg. Physical examination of the chest revealed many sonorous and sibilant râles throughout both lungs, and moist râles were heard at the lung bases. The heart was enlarged to the left; the heart sounds were normal and no murmurs were audible. The liver was palpable 3 cm. below the right costal margin. There was moderate ankle edema.

Laboratory examination revealed normal blood count and normal sedimentation rate; blood urea, 32 mg. per cent; total protein, 6.6 gram per cent; albumin, 2.5 gram per cent; glob-

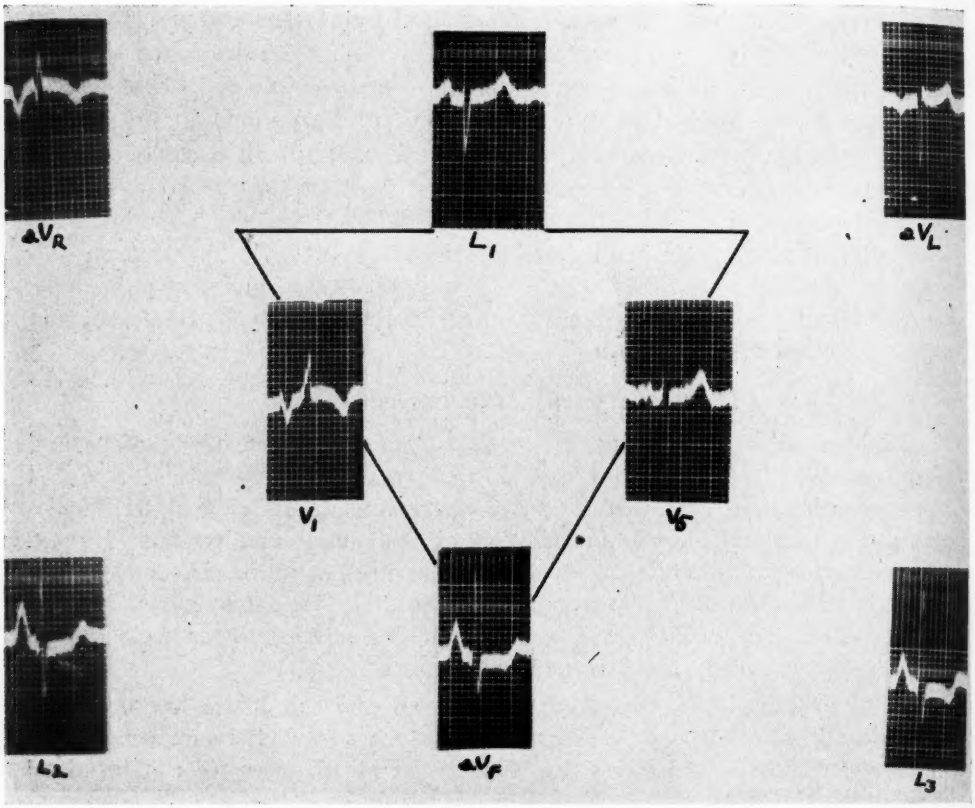

Fig. 1.—The electrocardiogram of Case 1 shows peaked and tall P waves in L_2 and L_3, right axis deviation in the standard limb leads, qR complex in aVR, tall and notched R in V_1, and a deep S in V_5. This tracing indicates right ventricular strain.

ulin, 4.1 gram per cent. Urinalysis showed albumin (++), a few hyaline cylinders, and many white and red cells. The chest roentgenogram showed enlargement of the left ventricle; the hilar markings were markedly increased, especially on the right side. The electrocardiogram exhibited P-pulmonale and right ventricular strain (Fig. 1). Pulmonary functions could not be obtained at admission because of the severe respiratory distress.

A diagnosis of bronchiectasis, pulmonary emphysema, bronchial asthma, chronic glomerulonephritis, cor pulmonale and congestive heart failure was made.

Following ten days of treatment with bronchodilators, oral digitalis, and mercurial diuretics, the patient lost 8 kilograms in weight. His condition improved, making pos-

sible the performance of pulmonary function studies. The vital capacity was 68 per cent and the maximum breathing capacity 42 per cent of the predicted values. The arterial oxygen saturation was 83 per cent. ACTH gel, 20 mg. twice daily, was started and given for seven days. Following this treatment, there was marked improvement in the pulmonary function tests; the vital capacity rose to 93 per cent, the maximum breathing capacity to 64 per cent, and the oxygen saturation in the arterial blood was 96 per cent. The dyspnea was relieved completely. During the treatment with ACTH the patient did not gain weight, and no other harmful effects of the drug were seen. He was discharged in a markedly improved condition.

One month later the patient was readmitted in severe status asthmaticus. There were no signs of right-sided heart failure. He coughed and produced a large amount of purulent sputum. The sputum culture revealed *Bacterium coli* and *Staphylococcus albus*, which were sensitive to all antibiotics. Pulmonary function tests on the fourth day of hospitalization revealed a vital capacity of 48 per cent and maximum breathing capacity of 23 per cent of the predicted values; the arterial oxygen saturation was 71 per cent and the carbon dioxide content was 51.04 volume per cent. Following treatment with antibiotics and intravenous infusions of 5 mg. ACTH daily for nine days, complete relief from asthmatic attacks was achieved. Repeated pulmonary function studies showed that the vital capacity increased to 97 per cent and the maximum breathing capacity to 71 per cent of the predicted values. The oxygen saturation of the arterial blood increased to 86 per cent and the carbon dioxide content decreased to 49.57 volume per cent. No undesirable effects of ACTH were observed, and the patient left the ward very much improved.

CASE 2.—M. Z., a 46-year-old man known to suffer from chronic bronchitis, bronchiectasis, pulmonary emphysema, and cor pulmonale, was admitted to the ward for the fifth time. His chief complaint was high fever of four days' duration accompanied by severe respiratory distress and productive cough.

On admission, the temperature was 38.8° C.; the respiratory rate was 28 per minute; the pulse was 96 per minute, regular; the blood pressure was 170/110 mm. Hg. Dyspnea, orthopnea, and marked cyanosis of the lips and nail beds were noted. There was slight clubbing of the fingers and marked engorgement of the neck veins. The chest was barrel-shaped and showed practically no respiratory expansion. There were hyperresonance and distant breath sounds, with a prolonged expiratory phase. Sibilant and sonorous râles were heard throughout both lungs, and coarse, medium-sized, moist râles were heard at the bases, especially on the right side. The heart sounds were barely audible. The liver was palpable 3 cm. below the right costal margin and was tender. There was no peripheral edema.

Laboratory Examination.—Red blood cell count was 6.8 million; hemoglobin, 17.5 gram per cent; white blood cell count, 18,900; differential count normal; hematocrit, 58 per cent. The blood sedimentation rate was normal. The venous pressure in the antecubital vein was 22.5 cm. H_2O; the circulation time (Decholin), 15 seconds. The urine was normal. Sputum culture yielded *Neisseria catarrhalis* and *Staphylococcus albus*, which were sensitive to antibiotics. X-ray examination of the chest revealed very prominent hilar markings, especially on the right side, and a reticular shadow in the lower half of the right lung. The electrocardiogram showed P-pulmonale and right ventricular strain. Pulmonary function studies, on admission, revealed a vital capacity of 32 per cent and maximum breathing capacity of 17 per cent of the predicted values. The spirogram showed a markedly prolonged expiratory slope. The arterial oxygen saturation was 55 per cent and the carbon dioxide content was 61.32 volume per cent. The patient was treated with antibiotics, bronchodilators, and xanthine preparations for fourteen days. Phlebotomy was performed on several occasions. At the end of this treatment, the temperature became normal and the general condition improved. The vital capacity rose to 50 per cent and the maximum breathing capacity to 25 per cent of the predicted values. The oxygen saturation in the arterial blood rose to 70 per cent and the carbon dioxide content was 66.67 volume per

cent. At this stage an intravenous drip infusion with 3 mg. ACTH daily was started and continued for six consecutive days. There was no further subjective improvement following this treatment. The pulmonary function test revealed vital capacity of 40 per cent and maximum breathing capacity of 19 per cent. The arterial oxygen saturation increased to 83 per cent and the carbon dioxide content decreased to 57.62 volume per cent. The patient's general condition was satisfactory and he was discharged.

The patient was readmitted one year later with a similar clinical picture. Signs of congestive heart failure were present. The vital capacity was 65 per cent and the maximum breathing capacity 15 per cent of the predicted. The arterial oxygen saturation was 83 per cent and the carbon dioxide content was 50.02 volume per cent. Digitalis, mercurial diuretics, and antibiotics were given. After a two-week period, repeated pulmonary function tests showed no significant change. Following eight days' treatment with 5 mg. ACTH daily by intravenous drip, the vital capacity was unchanged but the maximum breathing capacity increased to 28 per cent. The arterial oxygen saturation rose to 94 per cent and the carbon dioxide content was 53.25 volume per cent. The patient left the hospital in an improved state.

CASE 3.—M. M., a 65-year-old man, was admitted to the hospital complaining of increasing shortness of breath on slight exertion and productive cough for the last six years. On two occasions the patient noted swelling of the ankles which lasted for some days. He was a heavy smoker and an alcoholic up to two years prior to hospitalization.

On admission, a chronically ill patient was seen with orthopnea, dyspnea, and very marked cyanosis of the lips. There was no fever; respiration was 36 per minute, shallow. Fingernails showed a marked degree of clubbing. The pulse was regular, 100 per minute; the blood pressure was 135/100 mm. Hg. There was no engorgement of the neck veins in the sitting position. The chest was almost completely fixed in an inspiratory position. The lung fields were hyperresonant and the breath sounds were distant. Numerous sibilant and sonorous râles were heard all over the lung fields and some moist râles were found over the bases. The heart sounds were distant and no murmurs were heard. The liver was palpable 7 cm. below the right costal margin and was firm and tender. The spleen was not felt. There was no peripheral edema.

Laboratory Examination.—Red blood cell count was 7 million; hemoglobin, 19 gram per cent; white blood cell count, 7,300 with a normal differential count; hematocrit, 66 per cent. The sedimentation rate was 1 mm. per hour. In the sputum culture *Streptococcus anhemolyticus* and *Bacterium coli* were found. The venous pressure in the antecubital vein was 12 cm. H_2O, and the circulation time (Decholin) was twenty seconds. The electrocardiogram showed P-pulmonale and right ventricular strain; a deep Q wave in standard leads II and III and in aVF indicated healed posterior wall infarction. Chest roentgenograms showed pulmonary emphysema, increased hilar markings, and slight enlargement of the heart. Pulmonary function studies on admission showed vital capacity of 45 per cent and maximum breathing capacity of 16 per cent of the predicted values; in the spirogram a prolonged expiratory slope was seen. The oxygen saturation of the arterial blood was 83 per cent and the carbon dioxide content was 65.20 volume per cent. Cardiac catheterization revealed pulmonary artery pressure of 50/26 mm. Hg, mean 37.5 mm. Hg. The oxygen saturation in the brachial artery was 83 per cent, and in the pulmonary artery 54 per cent. The cardiac index was 2.17 liters per minute per square meter. The calculated pulmonary vascular resistance was 823 dynes second cm.$^{-5}$.

A clinical diagnosis of chronic spastic bronchitis, emphysema of the lungs, cor pulmonale, and congestive heart failure was made.

During the first five days of hospitalization, treatment consisted of antibiotics only. The clinical condition of the patient did not change markedly and although signs of congestive heart failure were still present, treatment with ACTH was started. The patient received 10 mg. ACTH daily by intravenous drip for five days and 20 mg. ACTH gel twice daily for three more days. A remarkable change in the clinical condition occurred.

The respiratory distress and the cough disappeared, the cyanosis diminished, the bronchial râles decreased, the liver became smaller and less tender, and the venous pressure dropped to 6 cm. H_2O. There was marked improvement in lung functions. The vital capacity increased to 59 per cent and the maximum breathing capacity to 26 per cent of the predicted normal values. The oxygen saturation in the arterial blood rose to 92 per cent and the carbon dioxide content dropped to 58.80 volume per cent. No gain in weight was noted. Three days after cessation of ACTH therapy the patient left the ward in a satisfactory condition.

Five months later he was readmitted in a state of severe congestive heart failure. The vital capacity was 54 per cent and the maximum breathing capacity was 17 per cent of the predicted values. The arterial oxygen saturation was 53 per cent and the carbon dioxide content was 65.36 volume per cent. Cardiac catheterization revealed a pulmonary artery pressure of 62/46 mm. Hg, mean 57 mm. Hg. Oxygen saturation of the pulmonary arterial blood was 23 per cent. The cardiac index was 2.44 liters per minute per square meter. The calculated pulmonary vascular resistance was 1017 dynes second cm.$^{-5}$.

Following treatment with antibiotics, bronchodilators, and mercurial diuretics for fourteen days, the respiratory distress and the cyanosis diminished. The signs of congestive heart failure subsided and the patient lost 10 kilograms in weight. The vital capacity increased to 77 per cent and the maximum breathing capacity to 31 per cent of the predicted values. The arterial oxygen saturation rose to 84 per cent and the carbon dioxide content dropped to 59.07 volume per cent. In order to investigate whether ACTH may produce further improvement of pulmonary function, the patient received 5 mg. ACTH by intravenous drip daily for seven days. There was no objective change in the patient's condition. The pulmonary function tests showed no significant change from those prior to this treatment. The patient was discharged in a satisfactory condition.

CASE 4.—D. J., a 65-year-old man suffering from diabetes mellitus and bronchial asthma for twelve years, was admitted to the hospital because of status asthmaticus of ten days' duration. He had been hospitalized three times previously because of upper respiratory tract infections, severe dyspnea, and cough.

On admission he was suffering from extreme dyspnea, orthopnea, and tachypnea. Cyanosis of the fingernail beds and the lips was noted. There was no fever; the pulse was regular, 112 per minute; the blood pressure was 145/85 mm. Hg. The chest was barrel-shaped and exhibited minimal respiratory movements. On auscultation, numerous sibilant and sonorous râles were heard throughout the lungs. The heart could not be outlined on percussion. The heart sounds were barely heard and no murmurs were audible. The liver was palpable 3 cm. below the right costal margin and was tender; the spleen was not felt. There was no peripheral edema.

Laboratory Examination.—Urinalysis revealed traces of albumin, sugar,$^{(+++)}$ no acetone; some leukocytes and erythrocytes were found in the sediment. The blood sugar was usually less than 180 milligrams per cent. The red blood cell count was 4.5 million; hemoglobin 13 gram per cent; white blood cell count, 11,000; the differential count was normal. The sedimentation rate was 3 mm. per hour. In the sputum culture, diffuse growth of *Staphylococcus aureus* was obtained, which was sensitive to antibiotics. A roentgenogram of the chest showed pulmonary emphysema and a heart of normal size. In the electrocardiogram, right ventricular strain was found.

A diagnosis of bronchial asthma, chronic bronchitis, emphysema of lungs, and cor pulmonale was made. Mild diabetes was present.

Pulmonary function tests could not be obtained on admission because of the severe respiratory distress. The patient received oxygen, digitalis, mercurial diuretics, aminophyllin, and Achromycin during the first four days. On the fifth day of hospitalization, pulmonary function tests showed that the vital capacity was 47 per cent and the maximum breathing capacity 20 per cent of the predicted values. The arterial blood oxygen saturation was 83 per cent and the carbon dioxide content was 48.01 volume per cent. Following

treatment with 10 mg. ACTH by intravenous drip daily for five days and 20 mg. ACTH gel for four more days, significant improvement occurred. The asthmatic attacks disappeared completely. The pulmonary function tests performed at that time revealed vital capacity of 87 per cent and maximum breathing capacity of 62 per cent of the predicted values. The arterial oxygen saturation was 84 per cent and the carbon dioxide content was 41.26 volume per cent. The blood sugar remained at the same levels as before treatment with ACTH. The patient was due to leave the hospital when, unfortunately, he was found dead in the lavatory. The cause of death remained unknown, as autopsy could not be performed.

TABLE I

CASE NO.	INITIALS, AGE, AND SEX OF PATIENT		VITAL CAPACITY (% OF PREDICTED)	MAXIMUM BREATHING CAPACITY (% OF PREDICTED)	OXYGEN SATURATION OF ARTERIAL BLOOD (%)	CARBON DIOXIDE CONTENT OF ARTERIAL BLOOD (VOL. %)
1	K. M. 35 M	Before ACTH	68	42	83	
		After ACTH	93	64	96	—
		Before ACTH	48	23	71	51.04
		After ACTH	97	71	86	49.57
2	M. Z. 46 M	Before ACTH	50	25	70	66.67
		After ACTH	40	19	83	57.62
		Before ACTH	65	15	83	50.02
		After ACTH	67	28	94	53.25
3	M. M. 65 M	Before ACTH	45	16	83	65.20
		After ACTH	59	26	92	58.80
		Before ACTH	77	31	84	59.07
		After ACTH	73	32	85	60.32
4	D. J. 65 M	Before ACTH	47	20	83	48.01
		After ACTH	87	62	84	41.26
5	A. E. 50 F	Before ACTH	67	—	88	49.61
		After ACTH	69	—	88	48.50

CASE 5.—A. E., a 50-year-old woman, suffering from respiratory distress for the past eight years, was admitted to the hospital because of severe shortness of breath, palpitation, cough, and fever. Eight months prior to admission, symptoms of congestive heart failure had appeared and treatment with digitalis was given.

On admission, the patient was severely dyspneic, cyanotic, and somnolent. The temperature was 38° C.; the pulse was regular, 120 per minute; the blood pressure was 130/70 mm. Hg. There was marked congestion of the neck veins. Respiration was shallow, 25 per minute; the respiratory movements of the chest were minimal. Hyperresonance, diminished breath sounds, numerous sonorous and sibilant râles all over the lungs, and fine, moist râles over the right base were found. The heart was enlarged in all directions. The heart sounds were normal; no murmurs were audible. The liver was palpable 5 cm. below the right costal margin; it was smooth and very tender. Marked peripheral edema was present.

Laboratory Examination.—Red blood cell count was 4.5 million; hemoglobin, 12.5 gram per cent; white blood cell count, 12,500; differential count, normal; hematocrit, 48 per cent. The blood sedimentation rate was 11/35 mm. In the sputum culture *Neisseria catarrhalis* and *Staphylococcus aureus* were found; they were sensitive to antibiotics. The venous pressure was 23 cm. H_2O. The chest roentgenogram showed increased hilar markings, the heart enlarged in all directions, increased density in the right lung base, and a small amount of fluid in the right costodiaphragmatic sinus. The electrocardiogram showed P-pulmonale and right ventricular strain. The vital capacity was 33 per cent of

the normal predicted value and a markedly prolonged expiratory slope was seen in the spirogram; the arterial oxygen saturation was 57 per cent, and the carbon dioxide content was 63.80 volume per cent.

The presumptive diagnosis was chronic bronchitis, infected bronchiectasis, obstructive emphysema, and cor pulmonale with congestive heart failure.

Treatment with digitalis, bronchodilators, antibiotics, and mercurial diuretics was given. In the course of five weeks a gradual improvement occurred. The signs of congestive heart failure diminished, the patient lost 10 kilograms in weight, and the chest x-ray revealed a decrease in heart size and clear lung fields. At this stage of the disease, the vital capacity was 67 per cent of the predicted value. The arterial oxygen saturation was 88 per cent and the carbon dioxide content was 49.61 volume per cent. In an attempt to improve the lung function further, ACTH gel, 15 mg. twice daily, was given for a week. The subjective condition of the patient did not alter, but she gained 4 kilograms in weight. The vital capacity and the arterial oxygen saturation remained unchanged. After the administration of a mercurial diuretic, she returned to her previous weight and left the hospital in a satisfactory condition.

COMMENT

It is generally agreed that obstructive emphysema is the main underlying cause in a large number of cases of cor pulmonale. Frequently a superimposed pulmonary infection aggravates this condition, causes a further decrease in the arterial oxygen saturation, and leads to respiratory acidosis. This course of events precipitates congestive heart failure in many cases of cor pulmonale.[10] Treatment should be directed primarily toward the underlying functional disturbance, the aim being to combat infection and open up intrapulmonary airways. Antibiotics and extensive use of bronchodilators have proved to be very effective. Adrenocorticotropic hormone has been shown to induce a marked and sustained bronchodilatation, probably by reducing the inflammatory and/or allergic swelling of the bronchiolar mucosa and by relieving bronchiolar spasm directly.[1-7] In cases of long-standing lung disease accompanied by cor pulmonale and hypoxemia, however, treatment with adrenocorticotropic hormone has been considered hazardous.[6, 8-10] Lukas[9] reported that in one patient with far-advanced lung disease and cor pulmonale the arterial oxygen saturation decreased after this treatment, indicating deterioration of respiratory function. Galdston and associates[8] reported deterioration of lung functions in three of five patients suffering from emphysema who received treatment with adrenocorticotropic hormone. These authors assumed that salt and fluid retention due to adrenocorticotropic hormone administration caused interstitial alveolar edema which interfered with blood oxygenation and caused an additional burden on an already insufficient myocardium. In both these studies, relatively large amounts of adrenocorticotropic hormone (40 to 240 mg. daily) were used.

Patient K. M. was admitted twice to our hospital in a state of severe pulmonary insufficiency and right-sided heart failure. The arterial oxygen saturation on the two admissions was 83 per cent and 71 per cent, respectively. After treatment with adrenocorticotropic hormone, dramatic clinical improvement occurred on both occasions. The arterial oxygen saturation increased markedly, together with the improvement of ventilatory functions. It is note-

worthy that the therapeutic effect in this case was the maximum in our series, which may be explained by the relatively young age of the patient and the short history of asthmatic attacks. It may be assumed that parenchymal changes in the lungs, besides the bronchiectasis, were of minimal degree and thus the pulmonary insufficiency was caused mainly by the bronchial obstruction.

Patient M. Z. had a long-standing history of chronic bronchitis and asthma, complicated recently by congestive heart failure. After two weeks of intensive treatment with antibiotics, bronchodilators, and mercurial diuretics, the signs of congestive heart failure diminished, but were still present when treatment with adrenocorticotropic hormone was started. Although following treatment the ventilatory functions did not improve (but even decreased somewhat), the arterial oxygen saturation rose from 70 per cent to 83 per cent, and the signs of congestive heart failure disappeared. On the second admission the same symptoms were present and similar observations were obtained. Arterial oxygen saturation increased this time to normal values, together with some improvement in the ventilatory functions.

Patient M. M., who showed features similar to the previous case, suffered from chronic bronchitis and asthma for many years. He was admitted in a state of severe congestive heart failure. After treatment with antibiotics and mercurial diuretics, the signs of heart failure diminished but they were still present when administration of adrenocorticotropic hormone was started. Following this treatment, the ventilatory functions increased and the arterial oxygen saturation rose from 83 to 92 per cent. The signs of congestive heart failure disappeared. On the second admission, however, treatment with adrenocorticotropic hormone was not followed by any improvement in the ventilatory functions and the arterial oxygen saturation did not change either.

Patient D. J. suffered for twelve years from chronic bronchitis and bronchial asthma. On admission he was in a state of severe asthma and had signs of pulmonary infection and right heart failure. During the first four days intensive treatment with digitalis, mercurial diuretics and antibiotics did not influence the patient's severe condition. Thereafter he was given treatment with adrenocorticotropic hormone, which was followed by complete relief from asthmatic attacks and regression of the signs of heart failure. Although his ventilatory functions improved markedly, no change in the arterial oxygen saturation was found.

Patient A. E. was admitted in an extremely severe state of congestive heart failure. She had a history of asthmatic attacks for the past eight years. After five weeks of intensive treatment with digitalis, antibiotics, and aminophyllin, the signs of congestive heart failure disappeared. There were still oxygen unsaturation and ventilatory insufficiency when an attempt was made to improve the lung functions further by treatment with adrenocorticotropic hormone. Following this treatment, no change in the ventilatory functions or oxygen saturation was observed. Signs of congestive heart failure did not reappear.

Our observations indicate that treatment with small doses of adrenocorticotropic hormone in a group of patients with cor pulmonale, characterized by marked decrease of ventilatory and respiratory functions, was of benefit and caused no harmful effects. The ventilatory functions improved in five instances, remained unchanged in two, and decreased slightly in one. The administration of adrenocorticotropic hormone was followed by marked increase of the arterial oxygen saturation in five of the eight treatments. In no instance did a decrease in the arterial oxygen saturation occur. It may be concluded that no disturbance of gas exchange occurred in our cases as a result of the treatment, as has been reported by Lukas[9] and by Galdston and associates[8] in patients suffering from similar lung diseases. These authors used greater amounts of adrenocorticotropic hormone in their series than we did. It appears, therefore, that the smaller amount may have a full beneficial therapeutic effect on the bronchial tree without causing interstitial alveolar edema, which latter is assumed to lead to a further aggravation of the pre-existing respiratory insufficiency.

SUMMARY

Ventilatory functions and arterial blood gases were studied on eight occasions in five patients with cor pulmonale before and after treatment with small doses of adrenocorticotropic hormone.

Marked ventilatory insufficiency and arterial oxygen unsaturation were present in all patients previous to treatment. Following treatment, ventilatory functions improved markedly in five patients, remained unchanged in two, and decreased slightly in one. The arterial oxygen saturation increased in five, and remained unchanged in three instances. No depression of gas exchange was observed.

It is concluded that in patients with cor pulmonale small doses of adrenocorticotropic hormone may be therapeutically effective without causing harmful side effects.

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HISTOCHEMICAL VARIATIONS OF THE LIPIDS OF THE ADRENALS IN GUINEA PIGS WITH EXPERIMENTAL ASTHMA

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IN EXPERIMENTAL[1-3] and clinical works[4-5] it is recognized that allergic phenomena may act as stress agents, with repercussions on the adrenal glands. Winer, Beakey, and Segal,[4] in autopsies of four persons who died in a state of asthma, found a discreet decrease of the lipids of the adrenals. Considering that the normal and pathologic function of the adrenal gland, as far as the production of steroid hormones is concerned, is intimately related to the content of lipids, especially of cholesterol and its esters, we have studied the modifications in the lipids of adrenals of guinea pigs subjected to prolonged and repeated attacks of experimental asthma. The presence of vitamin C was studied by histochemical methods, since, according to Deane and Morse,[6] its presence might be related to the capacity of the adrenal glands to synthesize the cortical steroids.

We chose guinea pigs because they are very suitable for the study of experimental asthma, for the anatomic-pathologic alterations of their shock organs are similar to some of the alterations found in the allergic human organism.[7-8]

The modifications thus obtained in the adrenal glands of the guinea pigs were compared to the alterations suffered by the same glands in other guinea pigs treated with formol, a very powerful stress agent. In this species, as Croxatto and Chiriboga[9] have pointed out, besides other alterations, there is a marked decrease of adrenal lipids, which they demonstrated in preparations stained with hematoxylin and eosin.

MATERIALS AND METHODS

We used forty-six male guinea pigs, weighing 250 to 350 grams, twenty of which we kept as controls. The entire group was fed grass without restrictions. Ten had been sensitized with a daily intraperitoneal injection of 10 per cent egg albumin for one week; fifteen days after the last injection they had been sprayed with the same albumin solution, as we have described in a previous paper,[10] for one hour every day for one to eight days. All of them had attacks of experimental asthma of variable intensity, and one hour after the last spraying they were sacrificed by a blow on the head.

Five other guinea pigs were sprayed with 0.05 per cent histamine, according to the method we have described in a previous paper,[11] every day for one to eight days. All animals went into shock. They were all killed one hour after the last spraying.

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Finally, the remaining ten animals were injected subcutaneously with 2 per cent formol. Three of them were sacrificed after the eighth injection, but the others died spontaneously; one after eight injections, another after six, three after the fifth inoculation, and two after the fourth injection. Almost all of them showed edema at the site of the injections, some with scars, a general bad state, and loss of weight.

A part of each of the adrenal glands of nearly all these animals (except those shocked with formol that died spontaneously) was fixed, after the death of the animals, in a solution of absolute ethanol, acetic acid, and distilled water saturated with silver nitrate for the histochemical study of vitamin C following the technique of Deane and Morse.[6]

The remaining parts of the adrenal glands of these animals were fixed in 10 per cent formol. One part was treated with the common histologic methods (paraffin inclusion and hematoxylin and eosin), and another part was cut on the freezing microtome for the histochemical study of lipids in general with Sudan III, the study of cholesterol esters by Schultze's method, the study of free cholesterol by Brunswick's method, the study of fatty acids and neutral fats by Lorrain's method, and the plasmal reaction by Feulgen and Vogt's method. To apply these methods, we followed Lison's[12] suggestions.

RESULTS

Control Guinea Pigs.—The only modification in the adrenal glands, macroscopically, was the marked variability of size and weight. This was one of the reasons why the latter was not recorded, the other reason being the purpose of the work itself which was not really related to the morphology but to the histochemical variations of the lipids and vitamin C.

TABLE I

METHODS	CONTROL GUINEA PIGS			ASTHMATIC GUINEA PIGS			GUINEA PIGS SHOCKED WITH FORMOL		
	GLOMERULI	FASCICULI	RETICULAS	GLOMERULI	FASCICULI	RETICULAS	GLOMERULI	FASCICULI	RETICULAS
Sudan III	±	+++	+	±	++±	±	±	+	0
Schultze	±	+±	±	0	+±	+	0	+	0
Brunswick	0	++	±	0	++	+	0	+	0
Feulgen	0	+++	+	±	+++	+	±	+	±
Lorrain	0	+++	+	0	+++	+	0	++	0
Vitamin C	+	++	+	+	++	+	0	±	0

These results obtained in the three zones of the adrenal glands' cortex with the six methods that we followed are summarized in Table I, on which plus signs (+) mark the average intensity of positive reactions; plus-minus (±) very little intensity; one plus (+), little intensity; two plus (++), medium intensity; and three plus (+++), great intensity.

It may be observed that the amount of vitamin C was regular in the fasciculi and small in the glomeruli and reticulas, as well as in the lumen and around the capillaries. In the connective tissue of the capsule of the glands there appeared some small quantities of ascorbic acid. It became apparent

under the shape of granules of different sizes and disseminated on the cellular cytoplasm, rarely agglutinated around the nuclei. In some animals vitamin C also showed on the walls and lumen of some of the capillaries.

In the glomeruli the reactions for the lipids were negative or of very little intensity, whereas in the fasciculi the reactions for the total lipids (Sudan III) and fatty acids (Lorrain) and the plasmal reaction were of great intensity. Finally, in the reticulas all the reactions were of very little or little intensity.

The great intensity of reaction with Sudan III, especially on the fasciculi, can be observed in Fig. 1; this reaction holds a small part of the glomeruli, the whole of the fasciculi homogeneously, and some parts of the reticulas.

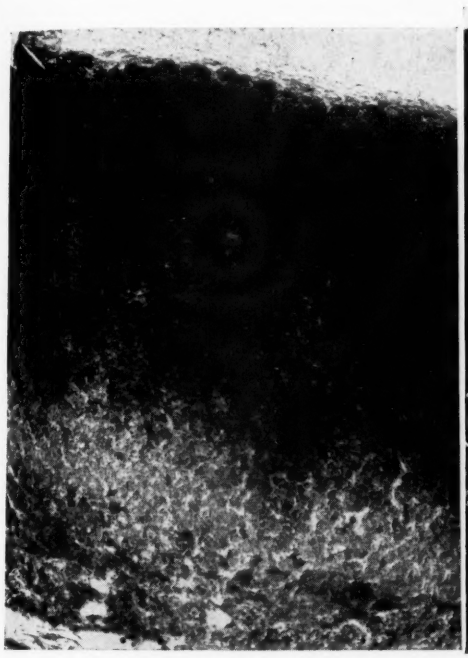

Fig. 1.

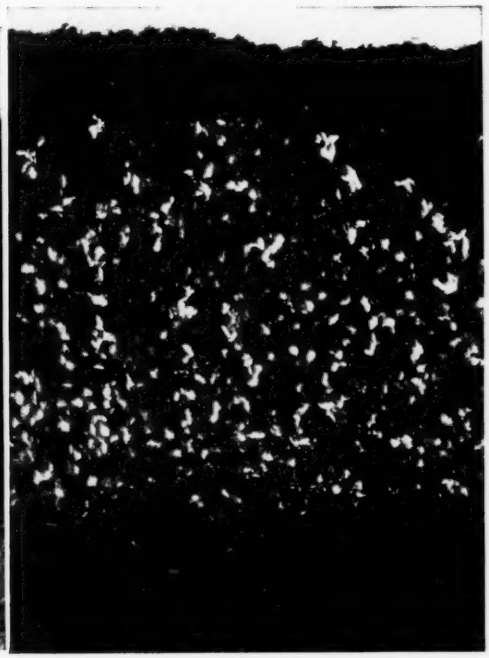

Fig. 2.

Fig. 1.—Control guinea pigs. Sudan III stain. Adrenal gland showing very intense reaction in the outer zone of the fasciculi and moderate reaction in the inner zone. Few cells react positively in the reticulas.

Fig. 2.—Same animal as in Fig. 1. Brunswick method. Birefringence of cholesterol intensively positive in the fasciculi.

Schultze's reaction showed variations in the different control animals, because in some of them the fasciculi are taken uniformly with great intensity and in others the reaction was uniform only in the exterior fasciculi with single islets on the interior fasciculi and in the reticulas. In others, besides those islets, there were some narrow bands of positive reaction spreading from the exterior fasciculi to the reticulas.

In the fasciculi and reticulas of some of the animals, Brunswick's reaction was of great uniformity and intensity but, in others, this reaction was visible only in single places on the fasciculi. The birefringence can be very well appreciated in Fig. 2.

Lorrain's reaction was of marked intensity in most of these animals, the red color for fatty acids predominating especially in the reticulas and fasciculi.

Feulgen and Vogt's reaction was intensely positive in all these animals, sometimes increasing its intensity in the interior fascicular zone.

Guinea Pigs With Experimental Asthma.—To establish the histochemical evidence of vitamin C and lipids, the same method as in the previous group was used, as shown in Table I. The distribution and intensity of the vitamin C reaction were similar to those observed in the control group.

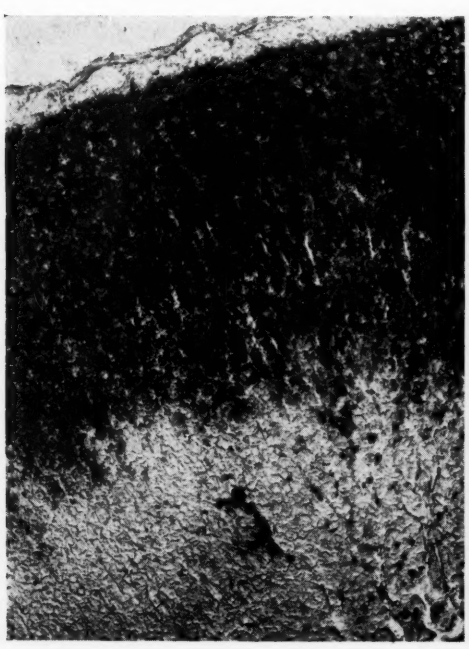

Fig. 3.

Fig. 4.

Fig. 3.—Guinea pigs with experimental asthma at the end of 8 days with aerosolized albumin. Sudan III stain. Moderate and sparse staining, especially in the outer zone of the fasciculi.

Fig. 4.—Same animal as in Fig. 3. Brunswick reaction. Moderate reaction in the fasciculi.

The results obtained in the glomeruli of these animals are similar to those of the control animals. On the fasciculi there was a discreet decrease in the intensity of reaction with Sudan III. The other reactions did not show any substantial change.

In those guinea pigs that suffered more intense attacks of experimental asthma during more days (as many as eight consecutive days), the reaction with Sudan III was diminished, but was positive and more intense on the exterior fasciculi (Fig. 3).

The intensity of Brunswick's reaction was not so positive in the same animals; this reaction took on a distribution similar to the previous one, as can be seen in Fig. 4.

We do not enumerate the results of the guinea pigs shocked with histamine because the variations are very similar to those shocked with albumin but, as there were only five animals, the variations may be of no importance.

Guinea Pigs Shocked With Formol.—The results of the methods that we followed were determined in the same manner as the two previous groups, and are included in Table I.

It can be observed that the very small amount of vitamin C present in the fasciculi of the sacrificed animals is on the walls and in the lumen of the capillaries. In the animals that died spontaneously we did not perform vitamin C determinations because, although they had been put in the freezer immediately after death, the autopsy was performed some hours later.

Fig. 5.

Fig. 6.

Fig. 5.—Guinea pigs shocked with formol by subcutaneous injection; end of the eighth day. Sudan III stain. Weak staining in the hypertrophied fasciculi.

Fig. 6.—Same animal as in Fig. 5. Brunswick method. Weak reaction in the fasciculi.

The reactions on the glomeruli are similar to those pointed out in the previous groups, whereas on the fasciculi a decrease of intensity in all the reactions is noticed, more particularly with Sudan III, Schultze, and plasmal reactions. The decrease of intensity of these reactions was obtained in the animals that died spontaneously, especially in those that died after five or more injections of formol. In such instances Sudan III was positive only in single places on the fasciculi (Fig. 5), very hypertrophic as can be seen more clearly in the sections colored with hematoxylin and eosin.

Schultze's reaction was completely negative in several of these animals; Brunswick's reaction was also remarkably less intense and it appeared only in small areas scattered in the fasciculi (Fig. 6).

Feulgen and Vogt's reaction was practically negative in one-half the animals of this group.

The average values of intensity of the employed methods are summarized in the table but, as we said before, there had been some variations of intensity among the animals of each group.

The average intensity of each of the reactions in the animals of each group is indicated in Chart 1, in which the vertical line represents the percentage of animals and the horizontal line the intensity of reaction of each technique separately.

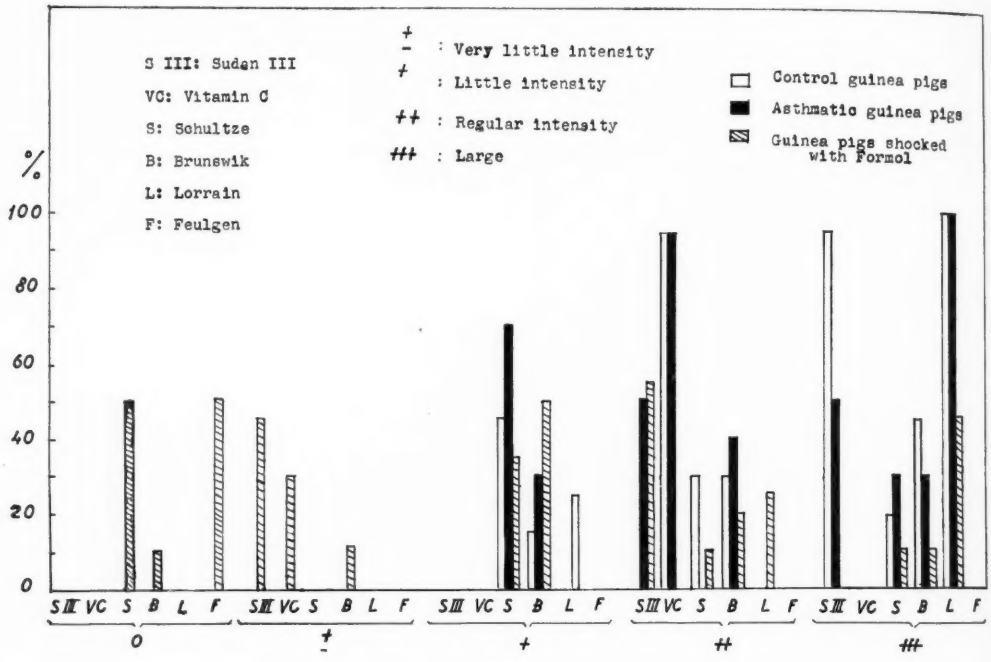

Chart 1.

In 100 per cent of the control and asthmatic animals there was a normal amount of ascorbic acid, whereas in the 30 per cent of the animals shocked with formol there was very little. As we explained before, it was impossible to determine whether or not vitamin C existed in the other 70 per cent of these animals.

It was observed that the reaction with Sudan III was very prominent in 100 per cent of the control animals, whereas this was the reaction in 50 per cent of the animals with experimental asthma and the reaction was of medium intensity in the other animals. On the other hand, none of the animals shocked with formol showed this same reaction with great intensity. We observed that in 55 per cent of them the reaction was moderate and in the other 45 per cent it was of very little intensity.

Schultze's reaction was of great intensity in only 25 per cent of the controls, against 30 per cent of the asthmatic animals and 10 per cent of those shocked with formol. It was moderate in 30 per cent of the control animals and in those shocked with formol. In 45 per cent of the control animals, in 70 per cent of the asthmatic animals, and in 33 per cent of those shocked with formol it was of little intensity. It was negative in 48 per cent of the latter animals.

Brunswick's reaction was of great intensity in 45 per cent of the controls, against 30 per cent of the asthmatic animals and 11 per cent of those shocked with formol.

In 30 per cent, 40 per cent, and 22 per cent it was of moderate intensity in the three groups of animals, respectively. In 15 per cent of the control animals it was of little intensity as compared to 70 per cent of the asthmatic animals and 48 per cent of the animals shocked with formol. In the latter, the reaction was of very little intensity in 11 per cent, and in the same percentage of animals it was negative.

Lorrain's reaction is similar in its variations to that of Sudan III. Feulgen and Vogt's reaction was negative in only 50 per cent of the animals shocked with formol.

DISCUSSION

According to Table I, the fasciculi of the adrenal glands in the three groups of animals is the place where the greater variations are produced in the average of intensity of reactions for the lipids with the methods employed.

When the results of the control animals are compared to those of the animals shocked with formol, the changes are more remarkable (Table I).

It can be observed that in the latter the intensity of all the reactions decreases, especially for the total lipids (Sudan III), for the cholesterol esters (Schultze's reaction), and the plasmal reaction (Feulgen and Vogt).

Instead, when we compared the asthmatic animals with the control animals we found only a moderate decrease in the average value of intensity of the total lipids. In turn, the intensity of reaction in the glands of these animals is much greater than the intensity obtained in the guinea pigs shocked with formol.

However, if we consider the number of animals in percentages we have the average of the animals that have more intensity of reaction with the six methods that we followed in each of the three groups.

So, the reaction for 100 per cent of the control animals is of great intensity for the total lipids, against 50 per cent of the asthmatic animals and the 10 per cent of the animals shocked with formol. It is also interesting to notice that in 70 per cent of the asthmatic animals the reaction is of little intensity for cholesterol and its esters, against only 45 per cent of the control animals and 45 per cent of those shocked with formol. The latter show even less intensity, that is to say, very little intensity (Chart 1).

Something similar happens with the free cholesterol, where only 15 per cent of the control animals have little intensity, whereas 30 per cent of the asthmatic animals had the same results. The guinea pigs shocked with formol comprised the group that had low intensity of reaction (48 per cent).

The intensity of reaction for vitamin C did not show any variation in the control or in the asthmatic animals, even in the case of guinea pigs with decrease of intensity for the lipid reactions.

On the other hand, in the three animals shocked with formol that were sacrificed, vitamin C was very reduced and with a characteristic distribution that, according to Deane and Morse,[6] exists in rats with intense and prolonged stress.

It is even more remarkable that the slight intensity of reaction for the lipids showed in the asthmatic animals, especially in those that suffered more intense attacks of asthma during more days (as many as eight consecutive days).

This decrease of the total lipids and of cholesterol and its esters would suggest a discharge or a decrease of the cortical steroids, because of a more intense glandular activity or because of a beginning of exhaustion due to the stress effect of the repeated attacks of asthma.

In fact, in the fasciculi of normal guinea pigs there is a fair amount of vitamin C, as well as great amounts of total lipids and cholesterol and its esters which are made evident because of the great intensity of Sudan III, Brunswick's, and Schultze's reactions (Table I, Figs. 1 and 2).

This already has been observed in normal guinea pigs by Stepto, Pirani, Consolazio, and Bell,[13] and by Vasconcelos Frazao.[14]

However, when these animals are treated with formol, which is a very powerful stress agent, after some time the negative image of the lipids disappears with hematoxylin and eosin, due to a loss of the glandular lipids.[9] As Eisenstein and Shank[15] said, the same happens when the deficiency of vitamin C acts as a nonspecific stress agent on these animals. In such circumstances, the intensity of Sudan III, Brunswick, and Schultze's reactions decreases because of the decrease of the cortical steroids,[13] as well as the amount of vitamin C.[6]

This decrease of the lipids took place in the guinea pigs shocked with formol (Table I, Figs. 5 and 6, Chart 1), especially in those guinea pigs that died spontaneously after several injections. Nearly the same thing, but with less intensity, happened in the asthmatic animals (Table I, Figs. 3 and 4), especially in those that had more intense attacks of asthma. This would suggest that the adrenal glands of these animals may be more active or at the beginning of exhaustion.[9]

Although the intensity of reaction for the lipids was reduced in the animals with experimental asthma, this was not so for vitamin C. In the animals shocked with formol and sacrificed after eight injections, however, a decrease in the reactions was obtained. It might be supposed, according to Deane and Morse[6] and Greep and Deane,[16] that the decrease of the lipids without the correlative decrease of vitamin C indicates an active secretion of the cortical steroids. This means that these animals would be in the resistance periods, whereas the decrease of lipids together with the decrease of ascorbic acid would indicate[6-16] the incapacity of the adrenals to synthesize the cortical hormones, since Sayers, Sayers, Liang, and Long[17] state that the changes of cholesterol and vitamin C of the adrenal glands are associated with the formation and liberation of the cortical hormones.

These animals shocked with formol might be, then, at the beginning of the exhaustion period. To make it clear, however, it will be necessary to study the modifications of intensity of all these reactions in animals subjected during longer periods of time to the action of attacks of asthma.

CONCLUSIONS

1. The reactions are of great intensity for the total lipids, the cholesterol and its esters, for the fatty acids, and the plasmal reaction in the most of the adrenal glands of normal guinea pigs. The reactions for vitamin C are of medium intensity.

2. In most of the guinea pigs shocked with formol all these reactions are of less intensity.

3. The intensity of reaction for the total lipids is slightly decreased in 50 per cent of the guinea pigs with experimental asthma; the reaction for cholesterol and its esters decreased in 30 per cent and 70 per cent of the same animals. The reaction for vitamin C is not changed.

4. The decrease of intensity in all these reactions for the lipids with the persistence of vitamin C suggests a greater glandular secretion due to the stress action of the repeated attacks of experimental asthma.

5. To make this point more clear, however, it will be necessary to study new aminals with the same methods but to subject the animals for longer periods of time to the action of attacks of experimental asthma.

SUMMARY

With the histochemical methods for the total lipids, cholesterol and its esters, the fatty acids and the plasmal reaction, and vitamin C, we have studied their variations of intensity in the adrenal glands of forty-six guinea pigs.

In the adrenal glands of most of the control animals all these reactions were of great or medium intensity, but decreased considerably in intensity in the adrenals of nearly all the ten guinea pigs shocked with formol.

In the glands of 50 per cent of the ten animals with experimental asthma the intensity of reaction for the total lipids was slightly decreased. The reactions for cholesterol and its esters also suffered a slight decrease of intensity in the adrenal glands of 30 per cent and 70 per cent of this group of asthmatic guinea pigs. The reaction for vitamin C did not show any variation.

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SUCCESSFUL TREATMENT OF HERPES ZOSTER OPHTHALMICUS WITH ANTIHISTAMINIC THERAPY

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IN A communication to the editorial department published in the April 30, 1955, issue of the *Journal of the American Medical Association,* I described an effective and rapid treatment for the cure of oral canker sores. Briefly, the treatment is as follows: 10 mg. of Chlor-Trimeton malleate is injected parenterally and followed by 8 mg. of long-acting Chlor-Trimeton orally every four hours. This is continued for twenty-four hours after disappearance of the lesions.

The same treatment was applied to cases of herpes labialis. Dramatic improvement was noted in each case within twenty-four hours. In one case, a 35-year-old housewife was subject to frequent appearances of herpes labialis throughout the year. On three different occasions during the past years these repeated labial eruptions were treated in the described fashion. Each time the lesions practically disappeared within twenty-four hours, with residual crust formation.

On the basis of the success obtained in the latter instance with the intensive antihistaminic therapy described, the same treatment was applied to a case of herpes zoster ophthalmicus.

CASE REPORT

A 46-year-old married male office clerk began feeling right-sided, lancinating, frontooccipital pain and pains through the right eyeball. They appeared a week prior to the vesicular eruption on the forehead and right temporoparietal regions of his scalp. They were of sufficient intensity to interrupt the patient's sleep each successive night.

He appeared for examination and treatment on the day following the appearance of the eruption, complaining of an intense itching. Examination revealed the characteristic lesions of herpes zoster. They covered the described area and were abruptly delineated at the midline. In addition, a punctate lesion of the cornea of the right eye was noted at about 2 o'clock. Both lids of the affected eye were markedly edematous.

Following a parenteral injection of 10 mg. of Chlor-Trimetone malleate, the patient was placed on 8 mg. of the long-acting type every four hours, orally. The response within twenty-four hours was as follows: The edema of both lids subsided, the punctate lesion of the cornea disappeared completely, and the vesicular lesion of the scalp had dried up. The latter were no longer visible within three days.

Herpes zoster follows the distribution of the spinal nerves. It is also accompanied by changes in the spinal fluid. Unlike herpes simplex, one attack is followed by lifelong immunity. Both types are of viral origin, and both conditions responded dramatically to the same therapy. Admittedly, this report deals with only one unusual and specific case and is by no means conclusive. A plea is made, therefore, to other investigators to administer antihistaminic therapy in the manner described, not only in similar cases but in other diseases of viral origin as well.

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ALLERGY AND MULTIPLE SCLEROSIS

A Critical Review

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INTRODUCTION

MULTIPLE sclerosis continues to be a serious medical problem. Despite numerous investigations, no etiological agent has yet been discovered and not one authenticated cure has been reported.[1] It has been estimated that approximately 300,000 cases exist in the United States, as compared with 250,000 cases of chronic poliomyelitis. In 1949 it was reported that 1,379 deaths were due to multiple sclerosis, and yet this is from incomplete data. Furthermore, considering that 90 per cent of the patients who develop multiple sclerosis do so before the age of 45, during the most productive and fruitful years of life, the problem assumes special import for its socioeconomic implications.[2]

In the search for a specific etiological agent in multiple sclerosis, many causes have been implicated. These include infection (either viral[3] or bacterial[4]); metabolic disorders, such as disturbances in carbohydrate[5] or fat[6] metabolism; psychogenic factors[8]; cerebral vascular disease, such as localized vasoconstriction or scattered thromboses of venules in association with altered coagulability[9]; sludging[10]; and allergy.[11]

This article is concerned primarily with an analysis of one of the possible factors, allergy. It is my intent to review and evaluate experimental, pathologic, and clinical data implicating allergy and possibly to indicate new avenues of approach in estimating the true etiological relationship, if any, between allergy and multiple sclerosis.

In order to evaluate any possible etiological agent of a specific disease or any therapeutic measure to relieve it, it is necessary that the disease entity be clearly defined clinically. Here we are at once confronted with serious difficulty. Kurland,[12] for example, is of the opinion that we may be dealing with two or more disease entities displaying the multiple sclerosis *syndrome* and that the disease may be etiologically different in northern and southern climates.

The difficulty in clinical definition is reflected in the diagnostic difficulties. Although many procedures and tests have been devised for the diagnosis of multiple sclerosis, none has proved clinically useful.† As yet, the only way,

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†A recent, as yet unconfirmed, report by Saifer, Rabiner, Oreskes, and Volk[13] describes a diagnostic electrophoretic pattern (protein profile) observed in multiple sclerosis patients.

according to Schumacher,[1] that the diagnosis of multiple sclerosis can be made is on the basis of (1) clinical evidence indicating widely scattered, discrete lesions in various parts of the central nervous system and (2) a tendency for alleviation or remission. Another difficulty stems from the evolutionary nature of the disease; it may be many years before the clinical picture indicates multiple sclerosis.

Kurland[12] indicates that only post-mortem examination is a reliable method for making the correct diagnosis. In a review of 25,000 autopsies which he analyzed, thirty-three had a clinical diagnosis of multiple sclerosis, of which twenty-three were confirmed and ten had been misdiagnosed. On the other hand, eleven persons were discovered at autopsy to have had multiple sclerosis which had not been clinically diagnosed. Nathanson[14] reports that 15 per cent of 250 patients seen in a large multiple sclerosis clinic were misdiagnosed.

Finally, any evaluation of an etiological agent or therapeutic procedure must also consider the observations already mentioned—that multiple sclerosis occurs only during a limited span of years, sparing the very young and the old; that the colder climates definitely increase the incidence and death rate[15]; and that it has a peculiar evolution and a tendency for remission.

THE STATUS OF ALLERGY IN THE ETIOLOGY OF MULTIPLE SCLEROSIS

To incriminate allergy effectively as a cause of multiple sclerosis, certain criteria must be fulfilled; these have been indicated by Cooke[16]: (1) an allergic constitution, (2) a repetitive history of symptoms, (3) a history of unusual or excessive contact with an allergen, and (4) positive skin reactions which, if not specific as in other atopic diseases, at least indicate the allergic constitution of the patient. To these four, the reviewer believes, should be added a fifth, usually included in Cooke's postulates: that elimination of a suspected allergen, if extrinsic, should relieve the symptoms, and that deliberate reintroduction of the suspected antigen should reproduce them.

Although this latter criterion is of marked value in clinical allergy (and, in the case of certain food and drug reactions, is the only way of establishing relationship between an allergen and disease), it may not hold in a disease entity in which allergy is not the sole factor but is associated with one or more agents acting simultaneously, or in which the allergen is intrinsic and cannot be eradicated. Were this fifth criterion to be demonstrated conclusively in multiple sclerosis (and this has not occurred under controlled conditions), the case for an allergic role would be markedly enhanced and the search for specific allergens thereby intensified. It is significant that, with all the years of observation of patients by competent investigators, no report has completely fulfilled these criteria.

1. *Is There an "Allergic Constitution" Among Patients With Multiple Sclerosis?*—Approximately 10 per cent of the general population suffers from recognized allergic diseases. On the other hand, data on the incidence of multiple sclerosis in allergic patients are meager. In my experience during

twenty years of allergy practice, including attendance at several large clinics, I have encountered only one patient with multiple sclerosis who also had hay fever. If allergy, as we commonly use the term, were a substantial etiological factor, the allergist should see a greater number of such cases. The literature reflects, both quantitatively and qualitatively, the rarity of the clinical evidences of allergy's relationship to multiple sclerosis, despite excellent experimental studies implicating allergy. Studies of the association of known allergic disease with multiple sclerosis are discussed further in Part III.

2. *Repetitive History.*—Here a certain superficial parallelism between allergic diseases and multiple sclerosis can be shown, since one of the chief characteristics of multiple sclerosis is its periods of exacerbation and remission. However, it remains to be demonstrated that these exacerbations are due to exposure to specific allergens and the periods of remission due to lack of contact with such allergens. Furthermore, although multiple sclerosis and allergic disease are both characteristically periodic, the two disorders differ sharply in their patterns of attack. The periodicity of allergic disease is largely predictable, particularly when the allergen is known and extrinsic. Neither the onset of symptoms nor the occurrences of remissions and exacerbations are predictable in multiple sclerosis, however. In addition, while most allergic disorders are completely reversible, leaving no pathologic residues, the characteristic pathologic lesion of multiple sclerosis (scarring of the brain tissue) appears to be irreversible.

Thus, the analogy is not complete, even if it be argued that the parallelism between multiple sclerosis and allergy need not necessarily apply to the so-called atopic diseases (in which heredity and positive skin reactions are dominant factors), but rather to the collagen disorders.

3. *History of Unusually Excessive Contacts With an Allergen.*—As a precipitating factor, this is not substantiated by any of the observers who have studied multiple sclerosis from the point of view of allergy.

4. *Positive Skin Reaction.*—Jonez[17] makes no statistical analysis of the results of skin tests performed using such allergens as foods, inhalants, pollens, and molds. Ehrentheil, Shulman, and Alexander,[19] who offer the most data on allergy testing but use the conjunctival test rather than the skin test, consider positive reactions to be evidence of specific sensitivity rather than indicators of the hypersensitive state, as implied by Cooke.[16] (They do not necessarily imply that every positive reaction is of clinical importance.) Skin testing, therefore, has not been correlated with multiple sclerosis.

5. *Manipulation of the Allergen.*—The fifth criterion (namely, the deliberate *clinical* induction of signs of multiple sclerosis by or with the aid of a specific allergen) also has never been truly demonstrated. Ehrentheil and his coworkers[19] have made this attempt in twelve patients, eliminating and then deliberately introducing specific foods (based upon allergy tests), hoping thereby to obtain induced remissions and exacerbations. This, it is recognized, is

a difficult procedure in a disease characterized by spontaneous remissions and exacerbations. The evidence, although strongly suggestive in some patients, was not conclusive for the group.

Despite the foregoing negative evidence, however, there is a basis for considering allergy as a mechanism for the induction of multiple sclerosis. The available data come from the experimental, pathologic, and clinical studies which are presented in this article.

I. EXPERIMENTAL EVIDENCE*

The experimental lesion most closely resembling multiple sclerosis is that encountered in experimental encephalomyelitis (iso-allergic). This is a disorder which produces widespread demyelinating lesions in the central nervous system. Accordingly, many studies of this phenomenon have been made in order to shed light upon the possible etiology of multiple sclerosis.

Several approaches have been used. Most of the studies have been concerned with attempts to induce brain damage through injection of nervous tissue. Serologic studies have also been made in a search for a possible immune mechanism. Other research has dealt with infection, metabolic disorder, and circulatory impairment.

Von Pirquet,[24] in 1907, shortly after he had introduced the concept (and the term) of allergy, indicated that an allergic mechanism was probably involved in the production of the papule and areola and in the accelerated reaction following revaccination with vaccine virus. It was logical, therefore, for Glanzmann, in 1927,[25] to implicate allergy in the postvaccinal encephalitis then relatively common. Stuart and Krikorian[26] extended this concept to include the encephalitis occasionally seen following antirabic treatment, and Finley[27] later endorsed it. A series of experimental studies was undertaken, beginning in 1932 with the observation by Hurst[28] of paralysis without obvious pathologic findings in the brains of four of forty-three rabbits injected with normal brain tissue.[30] During the following years, Rivers and his associates[29, 30] did a series of studies with monkeys in which prolonged injections with heterologous brain emulsion produced neurologic signs and symptoms owing to marked brain damage. This work was confirmed in 1940 by Ferraro and Jervis,[31] who also produced encephalomyelitis in monkeys with injections of heterologous (rabbit) brain over long periods of time. The pathologic findings, although not exactly typical of multiple sclerosis, resembled it. Freund and McDermott[32] subsequently (in 1942) introduced the use of adjuvants to enhance antibody production and, with this new tool in the hands of the researcher, a series of animal experiments was performed in which encephalomyelitis could be induced inexpensively by an exceedingly rapid method. Thus, in 1946 Morgan,[33, 34] as well as Kabat, Wolf, and Bezer,[35-37] independently observed that homologous brain combined with Freund's adjuvants could be injected into monkeys, with demyelination following two or three injections. The histologic picture was not that noted by Rivers and associates[29] and by Ferraro and Jervis[31] in which giant

*Hurst[23] has written an excellent and detailed review of experimental demyelination to which the reader is referred. Only a limited review is presented here.

cells were seen, but was, rather, that of a more acute process. Kabat, Wolf, and Bezer[36] were successful with a single injection and noted that both homologous and heterologous brain tissues were effective. Ferraro and Cazzullo[38] indicated that by modifying the dosage of a specific antigen (using smaller amounts) a picture more suggestive of the chronic condition could be produced. It was soon revealed that this phenomenon could be produced in the guinea pig, rabbit, mouse, and dog.

Although the rat was refractory, Lipton and Freund[39] later succeeded in producing encephalomyelitis by intradermal inoculation of brain emulsion plus adjuvants. Certain strains of both the dog and the mouse were noted to be particularly susceptible; others were more resistant.[23] Olitsky and Yager[40] were successful in inducing experimental encephalomyelitis in mice. Experiments with brain emulsions from different animals indicated that, with the exception of the immature cerebrum of neonatal or fetal origin, brains of guinea pig, rabbit, man, goat, cow, and pig could produce encephalomyelitis. Extracts of frog or fish brain were ineffectual.[37] Lumsden[41] further showed, in 1949, that emulsions of homologous, peripheral nerve could produce the same phenomenon as emulsion of brain tissue.* With the exception of organ emulsion, as reported by Kopeloff and Kopeloff,[42] encephalomyelitis could not be produced by investigators using emulsions of any other tissue (for example, muscle, liver, spleen, or lung, heterologous or homologous).

The pathologic findings in experimental encephalomyelitis varied somewhat from animal to animal, and were affected by the amount of antigen injected, the number of injections given, and whether or not an adjuvant was used. Subsequently, Freund, Lipton, and Morrison[43] showed that changing the *Mycobacterium butyricum* in the adjuvant from one grown in glycerin to one not grown in glycerin modified the type of response and produced a chronic encephalomyelitis which more closely resembled multiple sclerosis.

In the earlier experiments, the induction of encephalomyelitis by injection of nervous tissue with and without adjuvants had been inconstant. With the technique subsequently developed by Lipton and Freund[44] and by Freund and his co-workers[45]—selection of the Hartley strain of guinea pigs and a single intradermal injection of the emulsion of brain and adjuvant—all the animals developed iso-allergic encephalomyelitis. Lee and Olitsky,[46] utilizing pertussis vaccine as an additional adjuvant, have markedly enhanced the experimental production of encephalomyelitis in mice.

The production of acute disseminated encephalomyelitis by injection of brain emulsion immediately raises the question of whether or not a form of autosensitivity may be involved. This was clearly answered by Kabat, Wolf, and Bezer,[47] who induced the phenomenon by injection of monkeys with emulsions from portions of the recipient's own brain previously removed by operation, along with the adjuvants.

*Kabat, in a personal communication, states that he and other investigators have failed to confirm Lumsden's observation.

The general conclusion was that the mechanism which most probably accounted for the experimental production of encephalomyelitis was an antigen-antibody reaction. According to Kabat, Wolf, and Bezer,[48] antibodies produced by the injected brain tissue passed into the circulation and across the blood brain barrier to react with antigen in the central nervous system, thus producing perivascular lesions resembling those seen in multiple sclerosis. The search for a specific antigen disclosed that this was organ (rather than species) specific, that it was most abundant in the white matter of the brain, and that it was not present in fetal and neonatal cerebrum. Experiments with a variety of fractions obtained from extracts of brain tissue revealed that the proteolipide (a new type of lipoprotein discovered by Folch and Lees in 1951) comprised the encephalitogenic activity of brain.[49] This has been confirmed by Waksman and his associates[49a] and by others.

Further immunologic studies have provided another approach to the problem. Bailey and Gardner[50] subjected rabbits to prolonged immunization with a heat-killed vaccine of *Pasteurella boviseptica* grown in an infusion broth prepared from rat brain. The resultant antiserum contained antibodies against both the bacteria and the brain broth. Guinea pigs passively sensitized with this serum were shocked anaphylactically by a challenging dose of broth of brain or emulsified medullated nerve given intravenously. No other extracts of organs duplicated this phenomenon. This study showed not only that brain tissue can be antigenic, but that the resultant antibodies can be passively transferred.

The possibility that an immune mechanism was involved arose also from the previous observations of Rivers and Schwentker,[30] of Brandt, Guth, and Muller,[51] and of Witebsky and Steinfeld,[52] all of whom had indicated that antibodies against brain tissue could be produced by injections of heterologous brain tissue. Schwentker and Rivers[53] had also reported the presence of such antibodies with the use of homologous brain tissue. Their work antedated the use of Freund's adjuvants,[32] and antibodies were produced more readily when the brain tissue was combined with pig serum, given with vaccine virus, or after the brain had undergone autolysis. The suggestion was made, therefore, that in postinfectious encephalomyelitidies, autogenous antigens, capable of formation of brain antibodies, were liberated and the antigen-antibody union produced further brain damage, that is, demyelination.[36]

Comment on similar investigative work with the kidney is appropriate here. Masugi,[54] in 1934 (later confirmed by Smadel[55]), produced glomerulonephritis in rats by injecting them with antikidney serum obtained by injection of rabbits with kidney emulsion of the rat. It was concluded that specific tissue sensitivity of the kidney was responsible for the glomerulonephritis. Schick[56] had postulated in 1907, on purely clinical grounds, that nephritis resulted from an antigen-antibody mechanism; this was indirectly confirmed by Masugi[54] and Smadel.[55] Direct confirmation came from Cavelti and Cavelti,[57] who produced a form of glomerulonephritis in rabbits by injection of homologous kidney extracts inoculated with hemolytic streptococci.

This awaits confirmation. Lange and his co-workers[58] have further developed this concept by the clinical application of a complement fixation test as an expression of autosensitivity.

The Burky phenomenon is another suggestive observation of autosensitivity. In the rabbit, Burky[59] succeeded in inducing sensitivity to its own muscle and lens tissues by the simultaneous injection of tissue and staphylococcus toxin. Swift and Schultz[60, 61] confirmed Burky's results and also were able to produce identical sensitivity by modifying Burky's procedure, either by previously sensitizing to horse serum or by inducing infection with a streptococcus organism.

In view of such demonstrations of autosensitivity (that is, with the kidney as antigen as well as the Burky phenomenon), it might be postulated that nervous tissue also may act as an antigen and produce an autosensitivity capable of producing demyelination. The possibility of such a mechanism in encephalomyelitis was considered by Kolb and Bolton,[62] Freund, Stern, and Pisani,[45] Kopeloff and Kopeloff,[42] Morgan[33] and Kabat, Wolf, and Bezer.[36] Their attempts, however, to produce demyelination by the injection of antiserums to brain tissue were unsuccessful. What Masugi[54] and Smadel[55] were able to do with kidney tissue these workers could not do with tissue of the brain.

Not only were complement-fixing brain antibodies demonstrated in experimental encephalomyelitis, but further studies indicated the presence, not of a single antibody, but of a variety of antibodies resulting from injections of brain tissue.[63] These antibodies were not species specific, since brain tissue derived from many sources produced the same results. Lumsden, Kabat, Wolf, and Bezer,[64] working with experimental encephalomyelitis, noted multiple complement-fixing antibodies (to brain and placental tissue) in some animals, but none in others, or present only as anticomplementary to one tissue. Since the antigen was complex, multiple antibodies were produced. Thomas, Paterson, and Smithwick[65] not only observed the presence of complement-fixing antibodies, but reported the presence of flocculating antibodies which they equated with the complement-fixing antibodies. There is, however, no apparent relationship between the concentration of antibodies with the production of symptoms and pathologic lesions. Antibodies may be present without disease, or disease without antibodies. It has been suggested, therefore, that the presence of antibody may be only coincidental to the disease and unrelated to its pathologic manifestations.[23] This view does not seem compatible with a more positive role for an antigen-antibody mechanism in view of the remarkable role that the adjuvants play in experimental encephalomyelitis. Since the use of adjuvants in less complex studies enhances antibody production remarkably, the same mechanism is presumably applied here. Indeed, a logical explanation for the mechanism of experimental encephalomyelitis is that of an antigen-antibody reaction.

In addition to the circulation of antibodies against brain tissue previously demonstrated, several investigators, including Lipton and Freund[44] and Kope-

loff and Kopeloff,[42] demonstrated the presence of tissue-sensitizing antibodies by skin testing. These positive skin reactions, however, were produced only with heterologous brain and were not clearly correlated with the production of nervous disease. Waksman and Morrison,[66] however, later induced tuberculin-like skin reactions (delayed type) which were said to correspond in degree and time to the production of encephalomyelitis.

Where antibody production was demonstrable, unsuccessful attempts were made to produce the disease in the untreated animal by means of passive transference. This included the passive transfer not only of serum but also of peritoneal exudates and spleen or lymph node washings[37] as originally described by Chase[67] in the passive transfer of tuberculin sensitivity. Kabat and his associates[37] explain this failure of passive transference by assuming a continuous fixation of antibody by the antigen present in the nervous tissue of the donor.

Ferraro, Roizin, and Cazzullo,[68] utilizing this principle of the fixation of antibody, reduced the morbidity and mortality of encephalomyelitis by injecting brain emulsion without adjuvant simultaneously with an injection of brain emulsion combined with adjuvant. Their explanation was that antibody produced by means of the adjuvant presumably was neutralized by the antigen without the adjuvant. Freund,[69] however, observed that antigens injected without the complete adjuvants result in a hyperimmune response; with the complete adjuvants, the response is hyperergic. His explanation is that some subtle, unexplained, qualitative change is brought about by use of the complete adjuvant. This theory is discounted by Fischel, Kabat, Stoerk, and Bezer,[70] who regard the differences as only quantitative; that is, the adjuvant merely provides a localized inflammatory area which enhances the production of the same antibodies as those obtained by injection of the antigen without adjuvants. The assumption of Ferraro and his associates,[68] that injection of brain emulsion merely introduces antigen, is therefore not adequate, since the condition is not static and antibody can be produced even without adjuvants. Are two types of antibodies produced (one immune and one hyperergic), and do they interfere with each other? It is acknowledged that prolonged use of brain tissue can also produce a hyperergic state.[29-31]

Further evidence of an antigen-antibody mechanism in experimental encephalomyelitis is seen in the prevention of this phenomenon by the use of sodium salicylate[71] and ACTH,[72] presumably by interference with an allergic reaction. Kabat, Wolf, and Bezer[73] believe that cortisone inhibits antibody formation by inhibiting tissue reaction to the adjuvant (including tubercle bacilli[70]) but that, once antibody is formed, it does not affect the subsequent reactions with brain antigen. This accounts for the equivocal clinical results of cortisone and ACTH as reported by Glaser and Merritt.[74] Garcia-Reyes and co-workers,[75] in Thorn's laboratory, observed no impairment of the hypothalamus-pituitary-adrenal cortex function in multiple sclerosis.

It is possible for the brain to react through a variety of allergic mechanisms. Thus, Davidoff and Kopeloff[76] and Davidoff, Seegal, and Seegal[77] pro-

duced sensitivity in the brain by initially sensitizing animals with horse serum and subsequently injecting horse serum intracerebrally to elicit an Arthus reaction in the brain.

The pathologic pattern of the acute Arthus phenomenon in the brain, while not that typically produced in experimental encephalomyelitis, is nevertheless closer histologically to it than to multiple sclerosis. Repeated injections of specific antigen (egg or horse serum) into the sensitized monkey brain produced some lesions which took the form of demyelination and exhibited giant cells and other features similar to those following multiple injections of brain suspensions. The investigators, Jervis, Ferraro, Kopeloff, and Kopeloff,[78] concluded that these changes were due to the action of brain-specific antibodies which were formed in response to a complex antigen composed of foreign protein united to a hapten derived from the myelin damaged by repeated intracerebral inoculations. This explanation of the changes produced by the Arthus reaction in the brain is thus brought somewhat closer to the observations which follow the use of adjuvants and emulsions. Further substantiation comes from Koprowski and Jervis,[79] who found that guinea pigs which were treated with heterologous brain emulsion and adjuvants and failed to develop encephalomyelitis reacted violently to intracerebral injections of a suspension of heterologous brain. Those which succumbed quickly revealed a typical Arthus phenomenon with hemorrhage and necrosis; those which survived over a long period of time showed areas of demyelination which were noted not only at the site of injection, but scattered throughout the central nervous system.

The possibility of still another antibody mechanism capable of producing encephalomyelitis has been reported by Jervis,[80] who utilized the "carotid syndrome" of Forssman. The tissues of the guinea pig normally contain the Forssman antigen. If a serum containing Forssman antibodies is slowly injected centripetally into a carotid artery, an antigen-antibody reaction is induced in the brain tissue, producing areas of demyelination. Injected intracerebrally, the same serum produces changes characteristic of the Arthus phenomenon. Thus, a variety of antigens, in addition to brain tissue, is capable of producing brain reaction resulting in either the acute hemorrhagic necrosis of the Arthus phenomenon in some properly sensitized animals or demyelination in others.

Evidence has also accumulated to indicate that antigen-antibody mechanisms are not alone in the ability to produce demyelination. For example, enzymes, toxic agents, and production of cerebral anoxia have been implicated by various investigators. Thus, Vogel[81, 82] produced demyelination in animals by injecting a large dose of a lipolytic enzyme. Whether the results were achieved through enzymatic action may be questioned. Since rather large doses of the enzyme were injected, primary toxicity alone can explain the demyelination. Morrison and Zamecnik[83] showed that myelin sheaths could be broken down by several different types of enzymes, such as cobra venom

or the alpha toxin of *Clostridium welchii*. They indicated that the chemical reactions involved differed and that demyelination was therefore not a single, chemical process.

It also has been shown that any agent producing anoxia, such as potassium cyanide, as demonstrated by Ferraro,[7] can induce lesions resembling multiple sclerosis and that these lesions can be modified by altering the dosage of the agent. Likewise, Hurst[84] showed that sodium azide could produce a similar response. Morrison[85] produced demyelination by deliberately subjecting his experimental animals to an atmosphere poor in oxygen. Here no antigen-antibody mechanism could possibly be involved. There is also evidence that, at times, poisoning with carbon monoxide, nitrous oxide, and barbiturates can produce similar lesions.[84]

Alteration of the circulation, another way of producing cerebral anoxia, has been used by Putnam[9a] in the experimental production of demyelination. It is his contention that venule thromboses are responsible for the characteristic picture of multiple sclerosis.

Although most authorities believe that viruses produce encephalomyelitis through an allergic mechanism, Hurst[23] suggests that the distemper seen in dogs is specifically due to the neurotropic action of the virus, since inclusion bodies could be demonstrated. Furthermore, preparations of diseased brain, when injected into healthy animals, reproduced symptoms. Here, then, is another distinct mechanism for the production of demyelination.

It is evident, therefore, that demyelination may be a response of nervous tissue to many noxious agents, including infection, disordered metabolism (potassium cyanide, sodium azide, etc.), impaired circulation of the brain, and allergy. To summarize the role of allergy in this process, Hurst[23] may aptly be quoted: "Thus although the postulated antigen and antibody have not finally been identified and the detailed mechanism by which the nervous lesions are produced awaits discovery, a good deal of circumstantial evidence favors the view that the encephalomyelitis following injections of brain tissue is the expression of an allergic reaction."

Assuming the validity of Hurst's statement, we then ask how this applies clinically to multiple sclerosis. Granted that an animal's brain can be sensitized and, as a result of an antigen-antibody reaction, can produce lesions resembling those of multiple sclerosis, must we assume an *exclusive* allergic mechanism in man when it has been demonstrated that nonallergic mechanisms (infective, anoxic, enzymatic) also may produce demyelination in animals? If what we produce experimentally in animals casts light on a like mechanism in the human being, then we must accept the possibility that demyelination in the latter can also be a nonspecific tissue response to *many* noxious agents, including allergy. Multiple sclerosis, therefore, may be a syndrome (as suggested by Kurland[12]), rather than a specific disease entity, and demyelination only one of the signs, the extent of which (or even its presence) may vary from case to case.

Pursuing further the possible role that allergy may play in multiple sclerosis, which is strongly suggested by the experiments cited, the question arises: "Allergic to what?" Shall we look for specific brain sensitivity, either homologous or heterologous? How shall we test for such sensitivity? Skin testing with brain antigen may not be of help, since only a few investigators have demonstrated skin-sensitizing antibodies in the experimental animal, and since no specific correlation has been shown between the presence of antibodies (skin-sensitizing, complement-fixing, or flocculating) and encephalomyelitis induced in animals.

Can we test by serologic methods for hypersensitivity to nervous tissue in multiple sclerosis? Studies by Sachs and Steiner[86] were suggestive but not completely affirmative. Those by Kolb[87] in a small series were completely negative. These findings were not surprising since, in the experimental animal,[64] the presence of complement-fixing antibodies was not correlated with the presence of encephalomyelitis. Further advances in this field may result from identification of a purified antigen, so that the precise antibody sought for can be detected. In this connection, the finding of encephalitogenic proteolipide[49, 49a] points to a more hopeful goal.

II. PATHOLOGIC EVIDENCE

If multiple sclerosis is an allergic disease, does the pathologic examination support this concept? The answer to this question must be preceded by an analysis of the pathology of allergy which can then be compared with the pathologic changes encountered in both human and experimentally induced demyelination.

To begin with, there is no agreement as to specific pathologic changes induced by allergic disease. The usual allergic disorder leaves no residue for the pathologist to examine. The process, in most instances, is highly kinetic and reversible. It is the unusual allergic reaction which is irreversible and associated with demonstrable pathologic changes. In the past, the lesions of allergy were associated with an inflammatory response, resulting in fibrinoid degeneration in which eosinophils were conspicuous and considered diagnostic; the plasma cell was subsequently implicated along with the eosinophil. Current opinion, however, holds the view that an allergic response may take any of several forms. Allergy may be reflected pathologically by hyperemia and edema (usually reversible); by an acute inflammatory response frequently associated with necrosis (the Arthus phenomenon); by vascular damage and fibrinoid degeneration, as seen in periarteritis nodosa and other collagen diseases; by purpuric reactions; and possibly by eosinophilic granulomatosis. Which of these entities, then, may be implicated in multiple sclerosis?

The demyelinating diseases may be compared, in a way, with the collagen diseases. Although each of the collagen diseases, when classic, is distinct and differs from the others, there is enough overlapping in some cases to make specific diagnosis difficult and to show the close relationship that exists among them. Likewise, Adams and Kubik,[88] in an excellent review of the pathology

of the demyelinating diseases, indicated the obvious differences between classic multiple sclerosis, acute disseminated encephalomyelitis, Schilder's disease, and acute necrotizing hemorrhagic encephalopathy, yet noted sufficient overlapping to link them. Indeed, Ferraro[11] and others hold that these diseases are all variants of a single pathologic affection.[90]

The pathologic findings in multiple sclerosis are not static but vary with the duration and severity of the disease, whether acute or chronic.[89] Thus, there is more perivascular infiltration in the acute form and more scarring and involvement of the axis cylinders in the chronic. Further, some cases of multiple sclerosis show findings suggestive of acute disseminated encephalomyelitis. This is of particular importance since the allergic theory is strongly supported by the evidence based on the experimental production of encephalomyelitis, presumably by an allergic mechanism.

The reports on pathologic findings from the various workers who produced experimental encephalomyelitis probably differed because of the differences in technique employed. Freund, Lipton, and Morrison,[43] for example, observed that incorporation in the adjuvant of an organism *(Mycobacterium butyricum)* not grown in glycerin (one would think a relatively slight modification) produced remarkable differences in the pathologic picture; the resulting, more chronic condition more closely resembled multiple sclerosis. The same effect was produced by Ferraro and Cazzullo[38] by reduction of the dosage of antigen. The pathologic findings produced originally by Rivers and his associates[29, 30] resembled those of Schilder's disease. Morgan and her coworkers[33, 34] and Kabat and his group,[35-37] on the other hand, produced pathologic changes closer to those of acute disseminated encephalomyelitis.[90] In the more acute allergic type of reaction, as seen in the induction of the Arthus phenomenon in sensitized nervous tissue, hemorrhagic edema and necrosis are dominant in the pathologic picture, while demyelination is minor. Thus, experimentally produced pathologic reactions overlap in a manner similar to that noted in man by Adams and Kubik.[88]

In considering the possible etiological agents capable of producing demyelination, Adams and Kubik[88] indicate that the pathologic findings eliminate infection, disordered metabolism, and thrombosis. They state: "The most common cause of perivascular infiltration is infection, but the other pathologic findings do not resemble those of any known infectious disease. Allergy is another recognized cause. In both infectious and allergic diseases, lymphocytes, plasma cells and histiocytes are believed to play a role in the formation of antibodies. . . . It may be stated that the morbid anatomy of the demyelinative diseases favors the allergic theory."

The pathologist and immunologist appear to agree, therefore, that allergy may be important in the production of demyelinating diseases, including multiple sclerosis. Now, what has the clinician to offer?

III. CLINICAL EVIDENCE

In sharp contrast to the numerous excellent experimental studies concerning cerebral allergy, clinical reports, particularly on the relationship of

allergy to multiple sclerosis, have been few. This is surprising, indeed, since as early as 1936 Kennedy[91] had implied such a relationship. Although he enthusiastically endorsed a relationship between allergy and diseases of the central nervous system, writing about it extensively,[92, 93] he was careful to point out that it is easy to throw into the category of allergy any disease entity which defies diagnosis. He stated[93]: "Many allergic encephalopathies are constantly being diagnosed as multiple sclerosis. However, encephalopathies and *sclerose en plaque* are like two baskets flung over the back of an ass owned by a lazy man. Into them every trouble maker is thrust, disposed of and hidden away." The implication is that there are many truly allergic conditions of the brain, particularly retrobulbar neuritis, which have, understandably, been mistaken for multiple sclerosis, since multiple sclerosis frequently begins with disturbances which cannot be differentiated from retrobulbar neuritis, per se. But, as Kennedy points out, one can have recurrent retrobulbar neuritis on an allergic basis over many years without the manifestation of multiple sclerosis. He has implicated allergy primarily because of "the periods of remissions and the remarkable reversibility of the acute crisis, the attack on the optic nerve, and the neglect of sensory pathways: all of these much resemble the phenomena produced by localized allergic edema which has come to attack the central nervous system."[93] This, of course, refers to the superficial parallelism discussed earlier. But if we apply such reasoning to the acute phase, which might resemble allergic edema, does the same parallelism extend to chronic forms of multiple sclerosis? It is possible, of course, for the acute phenomenon to be on an allergic basis and the chronic symptoms and signs to be due to damage to the nervous system resulting from such an allergic reaction. This was proposed by Jonez,[17, 94, 95] who considered the underlying mechanism to be urticarial in nature.

Kennedy notes that in the rare cases of acute multiple sclerosis seen at autopsy, the pathologic finding is one of perivascular infiltration. "These fluid areas are usually well absorbed coinciding, no doubt, with improvement of symptoms. Only later do some of these edematous sites become sclerotic like the scar tissue of an old wound." It should be stated, however, that evidence would indicate that many kinds of inflammatory reactions in the brain ultimately lead to the kind of phenomena observed in multiple sclerosis, namely, perivascular infiltration and demyelinization. No one will disagree with Kennedy when he implies that, clinically, the central nervous system can react on an allergic basis. His observations on paralysis resulting from allergy to horse serum are classical. But he was never able to demonstrate a typical case of multiple sclerosis which could be specifically labeled as due to allergy. Cooke,[96] who collaborated with Kennedy for many years, does not, in his text, report any case of multiple sclerosis as specifically due to allergy. (Indeed, none of the recent texts on allergy mentions multiple sclerosis.[97])

Davison,[98] in a comprehensive review of allergy of the nervous system, lists but two references suggesting a possible relationship between allergy and multiple sclerosis. He reports no case of his own.

One of the earliest clinical investigations of the possible role of allergy in multiple sclerosis was conducted by Baer and Sulzberger.[20] They studied forty institutionalized patients under the care of neurologists, thereby reducing doubt as to the correctness of the diagnosis. These patients were scratch-tested with approximately 100 allergens (foods, inhalants, pollens, etc.), and careful histories of thirty patients were obtained. The authors concluded that 33 per cent of the patients presented evidence of personal or familial atopic disease or positive wheal reactions to skin tests, or both. This was not a higher incidence of atopy than that seen in normal persons investigated by others in a similar fashion.[99] Baer and Sulzberger[20] suggested that a non-atopic type of allergy might conceivably be implicated. An interesting aspect of this study was the attempt to demonstrate antibodies in the spinal fluid of one patient with marked skin reactions by the use of the passive transfer technique of Prausnitz and Kustner.[100] Although no reagins comparable to those obtained from the serum of the patient were demonstrable, very weak positive reactions were obtained only within twenty-four hours after inoculation of fresh spinal fluid. This was suggestive of a very unstable antibody.

Jonez[17] was possibly the most enthusiastic of the investigators who implicated allergy as an etiological factor in multiple sclerosis. He stated: "The allergic theory is based on edema. The pathologic change is one of edema or urticarial wheals scattered throughout the entire central nervous system at various times. The skin and central nervous system have the same origin—ectoderm. It is therefore reasonable to assume that the central nervous system is subject to the same type of reaction." Presumably, these urticarial wheals create internal pressures upon nervous tissue, causing its destruction, since the reaction occurs within an enclosed bony cavity and no expansion is possible.

It should also be pointed out that urticaria of the viscera, including the central nervous system, has usually been associated with urticaria of a generalized nature, involving the cutis and subcutis. No such association between generalized urticaria and multiple sclerosis has been reported by any observer. Urticaria may rarely be limited to a single wheal, but it is usually generalized rather than localized and evanescent rather than persistent to the point of causing pressure necrosis. The edema theory suffers also from the fact that, even during an acute attack of multiple sclerosis, examination of neither the eyegrounds nor the spinal fluid has been suggestive of increased intracranial pressure. Although edema can produce compression of a nerve to the point of paralysis, it can do so only within the confines of the narrow spaces through which the nerves enter or leave the skull or spinal cord. It is doubtful that edematous pressure within the brain can produce multiple sclerosis in a similar manner.

Jonez also advocated the theory that in multiple sclerosis the allergic reaction is mediated primarily through an antigen-antibody mechanism in which histamine is released. For this reason, he treated his patients with histamine.[17, 94, 95] This use of histamine was originally initiated in 1944 by

Horton,[21] who stated, however, that this use of the drug in no way implicated allergy. Histamine, according to Horton, was useful for physiologic reasons in that it produced an increase in the cerebral circulation, sometimes as high as 750 per cent, but Jonez[17] considered it to have a twofold function, pharmacologic and antiallergic. Jonez is practically alone in the idea that histamine therapy is antiallergic therapy. If we assume the validity of the histamine release theory in multiple sclerosis, it should be anticipated that patients should show improvement with an antihistaminic agent, none of which has proved to be useful in the treatment of the disease.[17, 74] These various reasons, then, would seem to dictate the conclusion that no histamine, and possibly no allergy, is involved in multiple sclerosis.

What, actually, are the results achieved by "allergic management" and histamine therapy as advocated by Jonez? Two reports are available. In the first, in which 152 cases were reported, eighteen of the nineteen listed as acute responded to treatment.[95] If this is compared with the chart tabulating the results of treatment, we find that the best results were obtained in twenty-five subjects. It can be presumed, therefore, that only seven cases, other than the acute ones, showed unusually good results. This follows the general pattern, regardless of type of treatment; the acute cases do well even if untreated. In sharp contrast, a group of twenty-nine patients were listed as showing only slight improvement objectively, which would make it exceedingly difficult to indicate whether or not any type of treatment was efficacious. In addition, twenty-eight cases showed only subjective improvement. Thus fifty-seven patients (approximately one-third of the total) represent cases in which improvement is doubtful or difficult to assess and yet are cited as showing some kind of "improvement." If to this is added the balance of twenty patients who either showed no improvement, became worse, or died, the results are then not striking. Totaling the patients who are listed in the first three categories as improved, we have eighty-two patients, or approximately 50 per cent. This would not seem to compare favorably with the 40 to 60 per cent of patients who ordinarily do just as well without treatment.[104, 105]

As a matter of fact, it is not easy to analyze Jonez's work, since he did not outline criteria, as advocated by Alexander and associates,[106] for the measurement of improvement. In a second report,[17] covering 1,500 patients who were treated by "allergic management" along with histamine, curare, psychotherapy, and physiotherapy, no results of treatment were recorded and, therefore, no positive conclusions as to the merit of the "total push" treatment can be drawn. Furthermore, other reports of Jonez[107, 108] confirm the impression that psychological support and suggestion contributed greatly.

In the report of Horton and his co-workers[21] 102 patients were treated intravenously with 2.75 mg. of histamine in 250 c.c. of isotonic sodium chloride given daily. A minimum of 13 and a maximum of 300 treatments per patient were administered. Of the twenty-four acutely ill patients, eighteen showed clinical improvement estimated at 40 to 70 per cent. In contrast, the chronically ill patients responded poorly; only thirty-six of seventy-eight

showed improvement ranging from 20 to 95 per cent. No objective improvement was seen in forty-two patients, although they were subjectively improved.

A more thorough investigation of the possible role of food allergy in sixty-five cases of multiple sclerosis is that of Ehrentheil, Shulman, and Alexander.[19] The possibility that inhalant allergy or allergy to bacteria or viruses might be equally responsible is disregarded. The ophthalmic reaction was used since, according to the observations of the participating allergist (Shulman[109]), skin testing with foods gives notoriously poor diagnostic results. A delayed type of reaction was observed and reported by the patient to the physician. However well instructed and conscientious patients may be, it is hard to evaluate material so assembled. As a control, fifty normal persons were also eye-tested with four selected antigens frequently found to produce reactions in multiple sclerosis patients. According to these authors, oranges and the cereals (oats, wheat, and particularly rye) were unusually potent allergens, as revealed by positive ophthalmic tests; 77.8 per cent of their multiple sclerosis patients reacted to rye. It should be noted, however, that in one of the control groups allergic patients without multiple sclerosis, 69.6 per cent, reacted to rye. This is an unusually high incidence of reaction to an allergen and one wonders whether or not the allergen, despite precautions, was not unusually irritating. Only 38 per cent in a second control group of nonallergic patients showed positive reactions. With such a relatively high incidence of positive reaction in control allergic patients without multiple sclerosis, it is difficult to assess the significance of these positive ophthalmic reactions. In addition to the ophthalmic test, skin tests were performed by the scratch method. A comparison, however, between the positive reactions obtained by skin tests and by eye tests revealed low parallelism. This is inexplicable and throws further doubt on the validity of either form of testing in multiple sclerosis patients. The investigation, however, was not limited to ophthalmic testing but included a thorough allergic history on a standardized form, a complete dietary analysis of each patient's food intake on a standardized form, an examination of each patient's nasal mucosa, and skin testing (scratch) with common foods as well as pollens. The ophthalmic tests were performed with twenty-five common foods. Among forty-nine cases of multiple sclerosis, adequately studied, a personal history of allergy was obtained in fourteen cases and a family history of allergy was reported in nine additional cases; 24.5 per cent of these patients showed positive reactions by scratch tests. Taking all these factors into consideration, 38.8 per cent of these cases could be labeled as being atopic, based on history and skin testing.

The evaluation of improvement by Ehrentheil, Shulman, and Alexander was based on a rigid classification originally devised by Alexander and associates.[106] This permitted, with some degree of accuracy, a measurement of the results of the therapeutic procedure, including the elimination of specific foods, based upon the ophthalmic reaction. In twelve instances, the deliberate, temporary introduction of these foods resulted in temporary exacerbations of symptoms. Although the results following the elimination of specific allergens

were startling in some instances, the over-all results were disappointing. Of the forty-five patients placed on presumably allergen-free diets (based on testing), full remissions occurred in only three patients (6.7 per cent). In one of these, egg sensitivity may have had some relationship to the symptoms. Two patients (4.4 per cent) reported clinically significant remissions. Nine other patients (20 per cent) were classified as having shown clinically significant improvement. In one of these patients there was strong correlation between ingestion of rye and neurological symptoms. A total of 31.1 per cent of these patients showed improvement through allergic management, as compared with 68.9 per cent who showed no improvement on such a regimen. When the suspected foods were deliberately ingested (the crucial test for evaluating whether a given food is an allergen), reactions were obtained in only twelve instances. Furthermore, there is no indication that the feeding tests were repeated.

The authors[19] suggest that allergy may possibly be a nonspecific stress factor similar to that noted following intercurrent infections, trauma, or emotional upsets which also are considered to aggravate the course of multiple sclerosis. In their estimation, therefore, the role of allergy may be nonspecific. This view is supported in large part by the observations of Squier,[110] who states: "It has been my belief that inhalant or food allergy is not of primary importance in the etiology of multiple sclerosis. I think it is possible and even probable that a patient who does have multiple sclerosis and also has clinical manifestations of allergy may have an aggravation of symptoms or may have a period of remissions broken by an acute upset. I think, however, that such an untoward reaction is entirely secondary and any influence exerted on the course of the disease probably is the result of general rather than specific effect." Dr. Squier cites case reports, one in particular, where a patient who suffered from ragweed hay fever noted exacerbations of symptoms referable to multiple sclerosis during the hay fever season.

DISCUSSION

In reviewing the data presented by the allergist, immunologist, and pathologist, it must be concluded that allergy may be an important factor in the production of multiple sclerosis and possibly in other demyelinative diseases. The strongest evidence comes from the immunologists, who have produced a variety of encephalopathies which, under controlled conditions, show clinical and pathologic pictures resembling multiple sclerosis. This was done, under conditions favorable to the production of a hypersensitive state, to mature brain tissue, to peripheral nerve containing myelin, or to both. It must be recalled that the results were achieved under unusual circumstances (prolonged courses of injections with antigens of nervous tissue or by use of such antigens with adjuvants) unencountered in clinical experience.[29, 30, 33-37, 43, 44] While the fact that sensitization to nervous tissue was produced in the experimental animal does not necessarily indicate that the same may be achieved in man, it strongly suggests such a possibility. More evidence for human sensitization is presented

by the occasional case of encephalomyelitis encountered during the Pasteur treatment against rabies, in which phenolized virus is injected along with rabbit cord.[26] Here, too, we have unusual circumstances under which pathologic changes are produced in the central nervous system by exposure to nervous tissue. These untoward reactions occur too infrequently to explain the relative frequency of multiple sclerosis. We can merely conclude that an allergic mechanism is capable of producing demyelinative disease.

Immunologists have definitely implicated hypersensitivity to nervous tissue, heterologous or homologous, intimately associated with myelin, in the demyelinative process. The allergists, however, on rather tenuous grounds, have been looking for sensitivity in other areas—for example, to foods, inhalants, and molds. There seems to be no rationale for this belief, however, since the allergists have not shown that the ingestion of food produces anything resembling multiple sclerosis. It is agreed that the brain can become sensitized and that common foods such as eggs, wheat, chocolate, milk, and pork can produce a variety of cerebral disorders, such as retrobulbar neuritis, migraine, Menière's syndrome, epilepsy, angioedema, narcolepsy, paralysis, and disturbances in cerebration.[96, 98] These phenomena are encountered rather infrequently in practice as true expressions of allergy and are reversible. Except for retrobulbar neuritis, they can in no way be linked to multiple sclerosis other than as evidence that the brain can be an allergic shock organ.

The search for clinical evidence to incriminate allergy in multiple sclerosis is, therefore, vexing because it is doubtful that the relevant allergen would be of an extrinsic nature (except perhaps the ingestion of nervous tissue in the form of food). Since the experimental evidence suggests sensitivity to nervous tissue itself in the demyelinative diseases, it would be more logical to test with antigens derived from this source rather than from foods, inhalants, etc. As a corollary, more suspicion should be directed toward sensitivity to ingested brain and possibly to nervous tissue containing myelin when meat is eaten. (Fish may not concern us, since its brain tissue fails to produce encephalomyelitis experimentally.) Even if such sensitivity were to be demonstrated clinically, skin testing probably would be negative, since in experimental animals the presence of complement-fixing antibodies, rather than demonstrable reagins, has been shown.[64, 87]

A delayed tuberculin type of reaction to brain tissue possibly has been demonstrated by Waksman and Morrison.[66] Most investigators,[33-37, 44, 65] however, have reported no antibodies capable of passive transfer in either the serum or washings of lymph nodes or spleen and, therefore, positive skin test reactions are improbable.

Allergy may play several roles in multiple sclerosis, but probably not as envisioned by the clinician who equates multiple sclerosis with known allergic disorders such as asthma, hay fever, etc. The usual allergic diseases, as suggested by Squier[110] and by Ehrentheil, Shulman, and Alexander,[19] possibly modify or aggravate the symptoms of multiple sclerosis in those patients who

are unfortunate enough to have both diseases. It is possible that allergy to foods and inhalants may be one of several components which together may produce demyelination; this, however, has not been established.

This view is based upon the observations of Burky,[59] who showed that under certain circumstances autosensitivity can be induced by the injection of staphylococcus toxin. Swift and Schultz[60, 61] also showed that, although the staphylococcus toxin produced autosensitivity readily, infection and sensitization with nonhemolytic streptococci deliberately induced in the animal, or the induction of allergy to horse serum, evoked the same response. The observations of Rivers and Schwentker[30] on the induction of sensitivity to brain tissue by simultaneous injection of such tissue with pig serum or with vaccine virus, or after brain had undergone autolysis, may be another manifestation of the Burky phenomenon. Is it possible that multiple sclerosis is a form of autosensitivity induced in the human being by a pre-existing allergic state such as the Swift-Schultz modification of the Burky phenomenon? The clinical evidence, as yet, is insufficient to associate multiple sclerosis with usual allergic diseases. It is difficult to assume the development of autosensitivity on the basis suggested in the absence of pre-existing and well-recognized allergic states, such as hay fever, asthma, etc.

If we are to consider further the possible role of autosensitivity as an explanation for an allergic mechanism in multiple sclerosis, then it would seem pertinent to consider the role that infection may play in autosensitivity. Hypersensitivity has been shown to be developed after vaccine virus infection, as evinced by revaccination skin reaction; also after lymphogranuloma venereum infection, similarly detected by skin test. Influenza, measles, and herpes simplex viruses have also been implicated in inducing allergic states. Raffel[111] indicates, in addition, that "continued investigation will reveal a widespread occurrence of this form of reactivity, probably in all infections caused by viruses." Nor has multiple sclerosis been associated with recognized viral diseases.

Störtebecker,[4] on the other hand, has implicated common bacterial infections with staphylococci, streptococci, and coliform bacilli. He noted that such infections frequently preceded multiple sclerosis and that evidence of infection was suggested by studies of antistreptolysin and antistaphylolysin titers and coli agglutination reactions. Störtebecker considered infection to be a precipitating factor and suggested that bacterial sensitivity possibly is involved in the etiology of multiple sclerosis.

Infection, bacterial or viral, may be implicated in other ways. It can induce autosensitivity (Burky phenomenon), which presumably could affect supposedly nonallergic persons. This may cause damage to myelin either by direct infection of nervous tissue or, indirectly, by toxin action which might release antigens capable of producing autosensitivity (following the pattern suggested by Rivers' studies with autolysates of brain tissue). Jervis, Ferraro, Kopeloff, and Kopeloff,[78] who gave intracerebral injections of brain antigen,

postulated that damaged brain (from trauma induced by the injection) acted as a hapten to produce lesions in areas of the nervous system distant from the sites of injection.

Kolb[18] attempted to induce sensitivity to brain tissue by Burky's method, employing staphylococcus toxin with rabbit brain emulsion. None of the thirty-five rabbits thus treated showed the dermal hypersensitivity to lens protein demonstrated by Burky, nor were precipitins to brain tissue demonstrable. Needling of the brain of sixteen of the rabbits did not cause demyelination.[87]

Despite these negative results, the possible role of autosensitivity in the production of demyelination needs further study. Autosensitivity has been so implicated in the experimental induction of encephalomyelitis that it cannot be ignored. The overwhelming evidence from experimental studies suggests going beyond the question of whether autosensitivity to nervous tissue can produce demyelination to a consideration of whether this phenomenon occurs in man without the use of adjuvants.

Although the Arthus phenomenon, when experimentally produced in the brain, causes damage which might explain, in part, the demyelinative process, the mechanism involved (initial sensitization followed by direct intracerebral inoculation of the antigen) obviously cannot be reproduced clinically. Knowing that sensitization to myelin and to its degenerative products has been induced, we can assume that nervous tissue contains the antigen and, therefore, no intracerebral inoculation is necessary. The antigen awaits a mechanism for its release. The experimentally induced Arthus phenomenon in the brain may be considered a highly exaggerated reaction of what may possibly proceed, clinically and pathologically, at a much slower pace. The type of antibody commonly associated with the Arthus phenomenon is the precipitin which has not been constantly demonstrable, however, in experimentally induced encephalomyelitis. To incriminate the Arthus phenomenon, we must first discover the initial cause of the sensitization. Does it follow infection or damage to the central nervous system from other sources?

Still another allergic mechanism experimentally capable of producing an Arthuslike reaction in the brain is the carotid syndrome of Forssman,[86] which is based on the normal presence of antibodies in the organism, including the central nervous system, and the introduction of specific antigen which would have to be directed particularly toward the brain. For this mechanism there is no clinical parallel or explanation, and its relation to multiple sclerosis is exceedingly doubtful.

As yet there is no one explanation which encompasses all the facets of multiple sclerosis. We must go back, therefore, to the questions raised earlier: Why the difference in incidence associated with climate? Why the exacerbations and remissions? Why does the disease apparently spare the very young and the aged?

Allergic mechanisms cannot explain the first question, since the incidence of the recognized allergic disorders has no relation to climate. If, however,

Störtebecker's[4] theory of the role of infection is valid, it might account for the greater incidence of multiple sclerosis in the northern climates because of the increased incidence of infections with streptococci and staphylococci.

The allergic theory can explain the exacerbation and remission phenomena seen in multiple sclerosis (although there are other possible explanations, such as repeated infections or repeated exposures to anoxic or toxic agents). It might be presumed that it takes a certain period of time for antibody formation, during which interval no symptoms may be present (latency). Then, when sufficient antibody is produced to combine with antigen, an explosive-like reaction occurs (exacerbation), in which the antigen is neutralized by the antibody. This might be followed by a period of remission, during which antigen, liberated as a result of damage to nervous tissue, produces more antibodies and the cycle is repeated and self-perpetuated. This is seen in experimental animals, in which, after an injection with brain tissue, explosive-like reactions seem to occur, with paralysis, blindness, or other symptoms suddenly appearing and then abating and recurring.

Can allergy suggest why multiple sclerosis spares the young and the very old? The young may be spared since it has been demonstrated experimentally that only mature brain is capable of inducing encephalomyelitis. Here it may be added that very young mouse brain also fails to reveal proteolipide and this substance was shown by Olitsky and Tal,[49] and Waksman and associates[49a] to be the encephalitogenic moiety of the central nervous tissue.[103] Another possible factor is an incubation period, the exact duration of which cannot be estimated, which may be so long as to spare the very young. Both factors may be involved —time for maturation of the brain and time for development of antibodies. As for the aged, they are known, judging by the clinical diseases of allergy, to have a lessened tendency to hypersensitivity. The possibility of sex hormone influence is not to be overlooked, since multiple sclerosis rarely occurs before puberty and after menopause.

Since experimental evidence indicates that most animals can become sensitized to homologous nervous tissue, and since Rivers and Schwentker[30] have shown that autolyzed nervous tissue may act as an antigen without the use of adjuvants, it is possible, therefore, that any damage to the control nervous system may initiate a self-perpetuating autosensitivity. This postulates the possibility that anoxia, lipolytic action of enzymes, impairment of circulation, infection, or possible allergic cerebral or cerebrovascular reactions even to such common antigens as foods may be the first step in the initiation of a chain reaction involving autosensitivity. One minor reversible allergic reaction, theoretically, might damage the nervous system sufficiently to release antigen capable of inducing a self-perpetuating irreversible allergy—autosensitivity. Assuming the presence of two allergic mechanisms working in sequence, skin testing with the common antigens might, therefore, be of some value only in elucidating the first mechanism. The over-all implication of this theory, therefore, may be, as suggested by Kurland,[12] that multiple sclerosis is not a specific disease but rather a syndrome in which many etiological agents may be concerned and in which

several mechanisms may be involved—an initial damaging of the central nervous system with release of antigen associated with myelin, followed by autosensitization to nervous tissue.

SUMMARY AND CONCLUSIONS

Evidence presented by the allergist, immunologist, and pathologist has implicated allergy as a cause for multiple sclerosis. This evidence has been reviewed and the following conclusions seem justified:

1. It is possible to produce in animals a clinical and pathologic condition resembling multiple sclerosis, by injections with extracts or emulsions of homologous or heterologous brain or cord tissue, with or without adjuvants.

2. An allergic mechanism is apparently involved in the experimental production of encephalomyelitis. The antigen, not yet specifically identified, is intimately associated with myelin tissue. Homologous or heterologous nervous tissue may be antigenic. The use of Freund's adjuvants, pertussis vaccine, and selections of strains of animals accelerate the development of experimental encephalomyelitis.

3. The antibodies involved in experimental encephalomyelitis apparently are not capable of passive transfer. Thus far antibrain antibodies have been demonstrated repeatedly by complement fixation, and by precipitation or flocculation against brain antigen. No direct correlation has been shown between the appearance and concentration of these antibodies and the appearance of symptoms in the animal. It may be that the specific antibody still awaits disclosure.

4. Results of skin testing with the antigen from heterologous or homologous nervous tissue are inconclusive.

5. There is no complete correlation between experimentally induced encephalomyelitis and multiple sclerosis, which it resembles. Presumably, they both may be mediated through an allergic mechanism. In experimental encephalomyelitis the allergen is ordinarily extrinsic and is introduced by injection. In multiple sclerosis the mechanism remains obscure. Some evidence suggests autosensitivity, implying the release of a latent intrinsic allergen from brain tissue.

6. The central nervous system is subject to a variety of allergic clinical disorders, traceable, as a rule, to extrinsic allergens and producing, ordinarily, reversible symptoms. In contrast, multiple sclerosis, as suggested by experimental studies, would be an irreversible allergic reaction.

7. Experimentally, allergic reactions within the brain, such as the Arthus phenomenon or the carotid syndrome of Forssman, can produce demyelination, but these have no clinical correlation and indicate merely that allergy in the central nervous system may be a factor in demyelination.

8. The development of specific tissue sensitivity (autosensitivity) as an explanation for multiple sclerosis has been considered. No mechanism for the clinical development of such an autosensitivity has been elucidated, however.

9. Infection (viral and bacterial) has been suggested as a factor in the liberation of the antigen from nervous tissue and the induction of autosensitivity which is self-perpetuating. Possibly, cerebral allergy, due to an extrinsic cause, may initiate a similar mechanism.

10. Demyelination may be produced experimentally by mechanisms other than an antigen-antibody reaction. These include inflammatory reactions, cerebral anoxia, demyelinating enzymes, circulatory disturbances, and metabolic poisons (potassium cyanide, barbiturates). Multiple sclerosis, therefore, may be a syndrome due to a variety of causes, including an intrinsic type of allergy.

11. The pathology of multiple sclerosis is compatible with, but not specific for, an underlying allergic mechanism.

12. Clinical studies of the allergic aspect of multiple sclerosis have been few and inconclusive. An approach limited to a narrow concept of allergy (atopy, involving sensitivity to foods and inhalants, and giving immediate positive skin test reactions) has not been fruitful and is contrary to the evidence presented experimentally, which indicates that demyelination induced on an allergic basis is nonatopic and probably is a form of autosensitivity.

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Announcements

R. R. Coombs Named to Editorial Post

Dr. R. R. Coombs, Assistant Director of Research in the Department of Pathology, University of Cambridge, has become editor-in-chief of the *International Archives of Allergy and Applied Immunology.*

Wilkes College

Wilkes College, Wilkes-Barre, Pennsylvania, has established within the Department of Biology a research laboratory devoted to the study of experimental hyposensitivity and related immunologic studies. Sheldon G. Cohen, M.D., is serving as Director of the research project.

Book Reviews

Manual of Clinical Mycology. By N. F. Conant, Ph.D., D. T. Smith, M.D., R. D. Baker, M.D., J. L. Callaway, M.D., and D. S. Martin, M.D., ed. 2, Philadelphia and London, W. B. Saunders Company, 1954, 456 pages.

Practical Medical Mycology. By E. L. Keeney, M.D., Springfield, Ill., Charles C Thomas, 1955, 145 pages.

Both of these books offer the clinician valuable practical information on the diagnosis and treatment of mycotic diseases, a field with which the average practitioner has relatively little experience, and which is rendered formidable by cumbersome and often variable terminology and by a number of different organisms producing rather similar disease pictures. The book by Dr. Conant and his coauthors gives more complete coverage and is generously illustrated with 202 photographs showing clinical, pathologic, and mycologic aspects. Dr. Keeney's book gives a briefer account of the subject. It is illustrated with diagrammatic presentations of the chief features of each disease which are graphic summaries but fail to show the necessary details. The latter book contains a brief chapter on fungus spores as allergens, but this is less complete than the treatment of the subject in most of the larger textbooks on allergy.

Cough Syncope. By Vincent J. Derbes, M.D., and Andrew Kerr, Jr., M.D., Springfield, Ill., Charles C Thomas, 1955, 182 pages. Price, $4.75.

The syndrome of syncope with loss of consciousness during a paroxysm of cough, sometimes designated as laryngeal vertigo, is discussed in detail. The authors have collected 255 reported cases from the literature and added 35 observed personally. The condition is said to be most common in middle-aged men, usually the ones who smoke and drink rather freely. Obesity, emphysema, and arteriosclerosis are believed to be predisposing factors. The possible physiologic basis is discussed fully without definite conclusions. The authors favor a circulatory, as opposed to a neurogenic, mechanism, although tachycardia induced by a vagal reflex is believed to be a factor in some patients. The book contains a full bibliography and will be of interest to physicians treating patients afflicted with this syndrome.